

PCT

WORLD INTELLECTUAL
PROPERTY ORGANIZATION

INTERNATIONAL APPLICATION PUBLISHED

WO 9602552A1

(51) International Patent Classification 6:

C07H 19/00, 21/00, 21/02, 21/04, C07K
1/00, 14/00, 17/00, 16/00, C12Q 1/00,
1/68, G01N 33/53, 33/567, A01N 43/04,
A61K 31/70

A1

(43) International Publication Date: 1 February 1996 (01.02.96)

(21) International Application Number: PCT/US95/09145

(22) International Filing Date: 19 July 1995 (19.07.95)

(30) Priority Data: 08/276,919 19 July 1994 (19.07.94) US

(60) Parent Application or Grant

(63) Related by Continuation
US 08/276,919 (CIP)
Filed on 19 July 1994 (19.07.94)

(71) Applicant (for all designated States except US): CYTO-
CLONAL PHARMACEUTICS, INC. [US/US]; Suite 330,
9000 Harry Hines Boulevard, Dallas, TX 75235 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): TORCZYNSKI, Richard,
M. [US/US]; 14247 Shoredale, Farmers Branch, TX 75234
(US). BOLLON, Arthur, P. [US/US]; 13227 Cedar Lane,
Dallas, TX 75234 (US).

(74) Agents: HANSEN, Eugenia, S. et al.; Richards, Medlock &
Andrews, Suite 4500, 1201 Elm Street, Dallas, TX 75270-
2197 (US).

(81) Designated States: AU, BR, CA, CN, FI, JP, KE, KR, LK,
MN, MX, NO, NZ, PL, RU, UA, US. European patent (AT,
BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL,
PT, SE).

Published

With international search report.
With amended claims.

(54) Title: LUNG CANCER MARKER

(57) Abstract

The present invention discloses an isolated and purified nucleic acid sequence and corresponding amino acid sequence to a novel protein specific for human lung cancer cells. This gene is expressed at a much higher level in these cells than in normal lung cells, other normal tissues and other tumor cell lines tested. Also disclosed are three additional recombinant forms of this gene and protein, in the first two cases a membrane spanning region is removed and in the third case an amino acid is changed by *in vitro* mutagenesis. Also disclosed are nucleic acid probes for the detection of lung cancer cells from tissue biopsy and body fluids such as serum, sputum and bronchial washings. A method for expressing the antigen in a host cell and its subsequent use as an immunogen in antibody production for test applications is described. An ELISA test to measure shed antigen present in patient samples as well as an enzyme test to measure activity in specimens also is described.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

LUNG CANCER MARKER

TECHNICAL FIELD

The invention relates to genes and proteins specific for certain cancers and methods for their detection.

BACKGROUND OF THE INVENTION

Lung cancer is the most common form of cancer in the world. Estimates for the year 1985 indicate that there were about 900,000 cases of lung cancer worldwide.

5 (Parkin, et al., "Estimates of the worldwide incidence of eighteen major cancers in 1985," *Int J Cancer* 1993; 54:594-606). For the United States alone, 1993

projections placed the number of new lung cancer cases at 170,000, with a mortality of about 88%. (Boring, et al.,
10 "Cancer statistics," *CA Cancer J Clin* 1993; 43:7-26).

Although the occurrence of breast cancer is slightly more common in the United States, lung cancer is second behind prostate cancer for males and third behind breast and colorectal cancers for women. Yet, lung cancer is the
15 most common cause of cancer deaths.

The World Health Organization classifies lung cancer into four major histological types: (1) squamous cell carcinoma (SCC), (2) adenocarcinoma, (3) large cell carcinoma, and (4) small cell lung carcinoma (SCLC). (The
20 World Health Organization, "The World Health Organization histological typing of lung tumours," *Am J Clin Pathol* 1982; 77:123-136). However, there is a great deal of tumor heterogeneity even within the various subtypes, and it is not uncommon for lung cancer to have features of
25 more than one morphologic subtype. The term non-small cell lung carcinoma (NSCLC) includes squamous, adenocarcinoma and large cell carcinomas.

Typically, a combination of X-ray and sputum cytology is used to diagnose lung cancer. Unfortunately, by the
30 time a patient seeks medical help for their symptoms, the cancer is at such an advanced state it is usually incurable. *Cancer Facts and Figures (based on rates from*

NCI SEER Program 1977-1981), New York: American Cancer Society, 1986). Routine large-scale radiologic or cytologic screening of smokers has been investigated. Studies concluded that cytomorphological screening did not significantly reduce the mortality rate from lung cancer and was not recommended for routine use. ("Early lung cancer detection: summary & conclusions," *Am Rev Respir Dis* 1984; 130:565-70). However, in a subpopulation of patients where the cancer is diagnosed at a very early stage and the lung is surgically resectioned, there is a 5-year survival rate of 70-90%. (Flehinger, et al., "The effect of surgical treatment on survival from early lung cancer," *Chest*; 1992, 101:1013-1018; Melamed, et al., "Screening for early lung cancer: results of the Memorial Sloan-Kettering Study in New York," *Chest*; 1984 86:44-53). Therefore, research has focused on early detection of tumor markers before the cancer becomes clinically apparent and while the cancer is still localized and amenable to therapy.

The identification of antigens associated with lung cancer has stimulated considerable interest because of their use in screening, diagnosis, clinical management, and potential treatment of lung cancer. International workshops have attempted to classify the lung cancer antigens into 15 possible clusters that may define histologic origins. (Souhami, et al., "Antigens of lung cancer: results of the second international workshop on lung cancer antigens," *JNCI* 1991; 83:609-612). As of 1988, more than 200 monoclonal antibodies (MAb) have been reported to react with human lung tumors. (Radosevich, et al., "Monoclonal antibody assays for lung cancer," In:

Cancer Diagnosis in Vitro Using Monoclonal Antibodies.

Edited by H. A. Kupchik. New York: Marcel Dekker, 1988).

MABs for lung cancer were first developed to distinguish NSCLC from SCLC. (Mulshine, et al.,
5 "Monoclonal antibodies that distinguish nonsmall-cell from small-cell lung cancer," *J Immunol* 1983; 121:497-502). In most cases, the identity of the cell surface antigen with which a particular antibody reacts is not known, or has not been well characterized. (Scott, et al., "Early lung
10 cancer detection using monoclonal antibodies," In: *Lung Cancer*. Edited by J.A. Roth, J.D. Cox, and W.K. Hong. Boston: Blackwell Scientific Publications, 1993).

MABs have been used in the immunocytochemical staining of sputum samples to predict the progression of
15 lung cancer. (Tockman, et al., "Sensitive and specific monoclonal antibody recognition of human lung cancer antigen on preserved sputum cells: a new approach to early lung cancer detection," *J Clin Oncol* 1988; 6:1685-1693). In the study, two MABs were utilized, 624H12 which binds a
20 glycolipid antigen expressed in SCLC and 703D4 which is directed to a protein antigen of NSCLC. Of the sputum specimens from participants who progressed to lung cancer, two-thirds showed positive reactivity with either the SCLC or the NSCLC MAB. In contrast, of those that did not
25 progress to lung cancer, 35 of 40 did not react with the SCLC or NSCLC MAB. This study suggests the need for the development of additional early detection targets to discover the onset of malignancy at the earliest possible stage.

30 Carcinoembryonic antigen (CEA) is a frequently studied tumor marker of cancer including lung cancer.

(Nutini, et al., "Serum NSE, CEA, CT, CA 15-3 levels in human lung cancer," *Int J Biol Markers* 1990; 5:198-202).

Squamous cell carcinoma antigen is another established serum marker. (Margolis, et al., "Serum tumor markers in non-small cell lung cancer," *Cancer* 1994; 73:605-609.).

Other serum antigens for lung cancer include antigens recognized by MAbs 5E8, 5C7, and 1F10, the combination of which distinguishes between patients with lung cancer from those without. (Schepart, et al., "Monoclonal antibody-mediated detection of lung cancer antigens in serum," *Am Rev Respir Dis* 1988; 138:1434-8) Furthermore, the combination of 5E8, 5C7 and 1F10 was more sensitive, specific and accurate for identifying NSCLC when compared to results from a combination of the CEA and squamous cell carcinoma antigen tests. (Margolis, et al., *Cancer* 1994; 73:605-609).

Serum CA 125, initially described as an ovarian cancer-associated antigen, has been investigated for its use as a prognostic factor in NSCLC. (Diez, et al., "Prognostic significance of serum CA 125 antigen assay in patients with non-small cell lung cancer," *Cancer* 1994; 73:136876). The study determined that the preoperative serum level of CA 125 antigen is inversely correlated with survival and tumor relapse in NSCLC.

Despite the numerous examples of MAb applications, none has yet emerged that has changed clinical practice. (Mulshine, et al., "Applications of monoclonal antibodies in the treatment of solid tumors," In: *Biologic Therapy of Cancer*. Edited by V.T. Devita, S. Hellman, and S.A. Rosenberg. Philadelphia: JB Lippincott, 1991, pp. 563-588). MAbs alone may not be the answer to early detection

because there has only been moderate success with immunologic reagents for paraffin-embedded tissue. Secondly, lung cancer may express features that cannot be differentiated by antibodies; for example, chromosomal deletions, gene amplification, or translocation and alteration in enzymatic activity.

After the gene to the MAb recognized surface antigen has been cloned, cytogenetic and molecular techniques may provide powerful tools for screening, diagnosis, management and ultimately treatment of lung cancer. An example of a lung cancer antigen that has been cloned is the adenocarcinoma-associated antigen. This antigen, recognized by KS1/4 MAb, is an epithelial malignancy/epithelial tissue glycoprotein from the human lung adenocarcinoma cell line UCLA-P3. (Strand, et al., "Molecular cloning and characterization of a human adenocarcinoma/epithelial cell surface antigen complementary DNA," *Cancer Res* 1989; 49:314-317). The antigen has been found on all adenocarcinoma cells tested and in various corresponding normal epithelial cells. Northern blot analysis indicated that transcription of the adenocarcinoma-associated antigen was detected in RNA isolated from normal colon but not in RNA isolated from normal lung, prostate, or liver. Therefore identification of adenocarcinoma-associated antigen in lung cells may prove to be diagnostic for adenocarcinoma.

The cloning of CEA and the nonspecific crossreacting antigen (NCA) has allowed the development of specific DNA probes which discriminate their expression in lung cancer at the mRNA level. (Hasegawa, et al., "Nonspecific crossreacting antigen (NCA) is a major member of the CEA-related gene family expressed in lung cancer," *Br J Cancer*

1993; 67:58-65). NCA is a component of the CEA gene family in lung cancer and is also recognized by anti-CEA antibodies, especially polyclonal antibodies. Because of the crossreactivity, investigations to analyze CEA and NCA separately in lung disease had been difficult. The use of DNA probes determined that lung cancer cells fall into three different types according to their CEA and/or NCA expression by Northern blot analysis. Specifically, lung cancers expressed both CEA and NCA mRNA, only NCA mRNA, or neither mRNA. CEA-related mRNA expression was always accompanied by NCA mRNA expression and there were no cases of CEA mRNA expression alone. The separate assessment of CEA and NCA expression in lung cancers may be important in determining the prognosis of lung cancers because the antigens have been described as cell-cell adhesion molecules and may play a role in cancer metastasis.

Another method to detect the presence of an antigen gene or its mRNA in specific cells or to localize an antigen gene to a specific locus on a chromosome is *in situ* hybridization. *In situ* hybridization uses nucleic acid probes that recognize either repetitive sequences on a chromosome or sequences along the whole chromosome length or chromosome segments. By tagging the probes with radioisotopes or color detection systems, chromosome regions can be identified within the cell. Investigations using *in situ* hybridization have demonstrated numerical chromosomal abnormalities in samples from human tumors, including bladder, neuroectodermal, breast, gastric and lung cancer tumors. (Kim, et al., "Interphase cytogenetics in paraffin sections of lung tumors by non-isotopic *in situ* hybridization. Mapping Genotype/phenotype heterogeneity," *Am J Pathol* 1993; 142:307-317).

Fluorescence *in situ* hybridization (FISH) allows cells to be stained so that genetic aberrations resulting in changes in gene copy number or structure can be quantitated by fluorescence microscopy. In this technique, a chemically labeled single-stranded nucleic acid probe homologous to the target nucleic acid sequence is annealed to denatured nucleic acid contained in target cells. The cells may be mounted on a microscope slide, in suspension or prepared from paraffin-embedded material. Treating the chemically modified probes with a fluorescent ligand makes the bound probe visible. FISH has been used for (1) detection of changes in gene copy number and gene structure; (2) detection of genetic changes, even in low frequency subpopulations; and (3) detection and measurement of the frequency of residual malignant cells. (Gray, et al., "Molecular cytogenetics in human cancer diagnosis," *Cancer* 1992; 69:1536-1542).

Other molecular markers for lung cancer include oncogenes and tumor suppressor genes. Dominant oncogenes are activated by mutation and lead to deregulated cellular growth. Such genes code for proteins that function as growth factors, growth factor receptors, signal transducing proteins and nuclear proteins involved in transcriptional regulation. Amplification, mutation, and translocations have been documented in many different cancer cells and have been shown to lead to gene activation or overexpression.

The *ras* family of oncogenes comprises a group of membrane associated GTP-binding proteins thought to be involved in signal transduction. Mutations within the *ras* oncogenes, resulting in sustained growth stimulation, have been identified in 15 to 30% of human NSCLC. (Birrer, et

al., "Application of molecular genetics to the early diagnosis and screening of lung cancer," *Cancer* 1992; 52suppl; 2658s-2664s). Patients with tumors containing ras mutations had decreased survival compared with patients whose tumors had no ras mutations. Polymerase chain reaction (PCR) amplification of ras genes can be analyzed to determine the presence of mutations by several methods: (a) differential hybridization of ³²P-labeled mutated oligonucleotides; (b) identification of new restriction enzyme sites created by the activating mutation; (c) single-strand conformational polymorphisms; and (d) nucleic acid sequencing. These methods combined with PCR technology could allow detection of an activated ras gene from sputum specimens.

Another family of dominant oncogenes, the *erb B* family, has been found to be abnormally expressed in lung cancer cells. This group codes for membrane-associated tyrosine kinase proteins and contains *erb B1*, the gene coding for the epidermal growth factor (EGF) receptor, and *erb B2* (also called *Her-2/neu*). The *erb B1* gene has been found to be amplified in NSCLC (up to 20% of squamous cell tumors), while the EGF receptor has been shown to be overexpressed in many NSCLC cells (approximately 90% of squamous cell tumors, 20 to 75% of adenocarcinomas, and rarely in large cell or undifferentiated tumors). (Birrer, et al., *Cancer* 1992: 52 suppl; 2658s-2664s). Amplification of the related oncogene *erb B2* (*Her-2/neu*) occurs infrequently in lung cancer but is a negative prognostic factor in breast cancer. However, overexpression of the *erb B2* protein product, p185^{neu}, occurs in some NSCLC and may be related to poor prognosis. (Kern, et al., "p185^{neu} expression in human lung

adenocarcinomas predicts shortened survival," *Cancer Res* 1990; 50:5184-5191).

5 A third family of dominant oncogenes involved in lung cancer is the *myc* family. These genes encode nuclear phosphoproteins, which have potent effects on cell growth and which function as transcriptional regulators. Unlike *ras* genes, which are activated by point mutations in lung cancer cells, the *myc* genes are activated by overexpression of the cellular *myc* genes, either by gene
10 amplification or by rearrangements, each ultimately leading to increased levels of *myc* protein. Amplification of the normal *myc* genes is seen frequently in SCLC and rarely in NSCLC.

15 The loss or inactivation of tumor suppressor genes may also be important steps in the pathway leading to invasive cancer. Tumor suppressor genes function normally to suppress cellular proliferation, and since they are recessive oncogenes, mutations or deletions must occur in both alleles of these genes before transformation occurs.

20 A phosphoprotein p53, which is encoded by a gene located on chromosome 17p, suppresses transformation in its wild-type state. While in its mutant state, p53 acts as a dominant oncogene. p53 functions in DNA binding and transcription activation. Mutations of p53 have been
25 found in many human cancers including colon, breast, brain and lung cancer cells. (Birrer, et al., *Cancer Res. (suppl)* 1992, 52:2658s-2664s). In NSCLC cell lines, p53 mutations have been found at a rate of up to 74%. (Mitsudomi, et al., "p53 gene mutations in non-small-cell
30 lung cancer cell lines and their correlation with the

presence of ras mutations and clinical features," *Oncogene* 1992; 7:171-180).

5 Despite all of the advances made in the area of lung cancer, medical and surgical intervention has resulted in little change in the 5-year survival rate for lung cancer patients. Early detection holds the greatest hope for successful intervention. There remains a need for a practical method to diagnose lung cancer as close to its inception as possible. In order for early detection to be
10 feasible, it is important that specific markers be found and their sequences elucidated.

15 A lung cancer marker antigen, specific for NSCLC, has now been found, sequenced, and cloned. The antigen is useful in methods for detection of non-small cell lung cancer and for potential production of antibodies and probes for treatment compositions.

BRIEF DESCRIPTION OF THE DRAWING

FIGURE 1 depicts the alignment of the amino acid sequence of HCAVIII with previously described carbonic anhydrases. Conserved amino acids are shown in bold.

SUMMARY OF THE INVENTION

The invention concerns a lung cancer antigen (HCAVIII) gene specific for non-small cell lung cancer.

5 In one embodiment, the invention relates to a substantially purified nucleic acid (SEQ ID NO:1) encoding the pre-protein sequence shown in SEQ ID NO:2.

10 In other embodiments, the invention relates to cDNAs which encode the mature form of the protein (SEQ ID NO:4), or a truncated form of the protein lacking the transmembrane domain (SEQ ID NO:13 and SEQ ID NO:15), or a protein in which one or more of the amino acids in the phosphorylation region have been altered to affect that function, an example of which is shown in SEQ ID NO:18.

15 In other embodiments, proteins encoded by the cDNA of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:12, SEQ ID NO:14, and SEQ ID NO:17 are provided.

In another aspect, the invention relates to a recombinant DNA clone for HCAVIII.

20 In further aspects of the invention, expression vectors for HCAVIII and modifications thereof are an object.

The invention further relates to methods of detecting lung cancer.

25 In one aspect an *in situ* hybridization technique is provided. In another aspect, a fluorescence *in situ* hybridization technique is provided. In a further aspect, an ELISA assay is provided. In another aspect, detection of carbonic anhydrase activity which correlates with lung cancer antigen is provided.

DETAILED DESCRIPTION OF THE INVENTION

The nucleic acid sequence coding for a cell surface protein (said protein hereinafter designated HCAVIII) which is highly specific for non-small cell lung cancer cells has now been obtained. This gene sequence will facilitate detection and treatment of the disease, which to date has often proven difficult.

The HCAVIII cDNA in the vector pLC56 has been sequenced and characterized including the entire coding region and substantially all of the upstream and downstream non-translated regions. The cDNA in pLC56 was sequenced on both strands from exonuclease III-generated deletions and subsequent subcloning into M13 vectors or directly from the cloning vectors using the di-deoxy method and a SEQUENASE® Version 2.0 kit (U.S. Biochemicals, Cleveland, OH). Additional regions of DNA were subcloned as small restriction fragments into the same vectors for sequence analysis. Overlapping segments were ordered using MacVector Align software (Kodak/IBI Technologies, New Haven CT). SEQ ID NO:1 represents the cDNA encoding HCAVIII and a presumed signal peptide. SEQ ID NO:2 represents the signal peptide (amino acid residues -29 to -1) followed by the mature protein (amino acid residues 1 to 325). As predicted from the cDNA sequence in pLC56, a protein of about 354 amino acids is encoded with the predictive size of 39448 daltons. A hydrophilicity plot (MacVector software, Kodak/IBI Technologies) of this protein provided strong evidence of a leader peptide at the N-terminus and a membrane-spanning segment near the C-terminus. The membrane-spanning segment provides evidence that this protein is membrane bound, as also predicted by its positive selection with

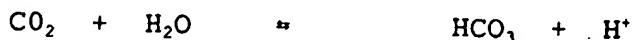
panning methodology (See Watson, et al., *Recombinant DNA*, 2nd ed., pp. 115-116, 1992). The cleavage site of the signal as predicted by von Heijne (von Heijne, Gunnar, *Nucleic Acids Res* 1986; 14:4683-4690) is 29 amino acids down from the N-terminus methionine. SEQ ID NO:3 corresponds approximately to the coding region of the mature polypeptide. The subsequent "mature" protein is proposed to be 325 amino acids, initiating with serine, and of a calculated 36401 daltons and a pI of 6.42 (SEQ ID NO:4).

Homology searches against NCBI BlastN or BlastX version 1.3.12MP (National Center for Biotechnology Information, Bethesda, MD) provided evidence the gene and protein are novel, not previously identified in either database. (Altschul, et al., "Basic local alignment search tool," *J Mol Biol* 1990; 215:403-410). Additional searches against another database (Entrez, version 9) gave similar results.

The isolation of a second cDNA encoding HCAVIII permitted the identification of new sequences within the 5'-and 3'-prime untranslated regions of this gene. SEQ ID NO:5, a cDNA encoding HCAVIII and a portion of the 5' and 3' nontranslated regions, has substantial identity with SEQ ID NO:1 (positions 1-1104 of SEQ ID NO:1 are identical to positions 85-1188 of SEQ ID NO:5). The encoded protein is listed in SEQ ID NO:6 and is identical with SEQ ID NO:2. Homology searches of NCBI BlastN against SEQ ID NO:5 showed these gene sequences have not been previously identified. SEQ ID NO:7 represents additional cDNA sequences of the 3' nontranslated region of the HCAVIII gene located downstream from the sequences depicted in SEQ ID NO:5. Homology searches against the same data base

identified two clones with homology to SEQ ID NO:7. Both sequences are expressed sequence tags (EST), the first EST04899 (345 bp) and the second HUMGS04024 (466 bp).

5 Alignment searches indicate this protein shares common features with the seven human carbonic anhydrase proteins previously identified. However, as described below, certain structural features distinct to HCAVIII exist that may confer unique properties to this protein and a role in the transformation pathway to tumorigenicity.
10 This group of enzymes catalyze the hydration of carbon dioxide



and in reverse the dehydration of HCO_3^- . This protein is identified as a carbonic anhydrase (CA) based on the
15 conservation of amino acids at positions critical for the binding of Zn^{+2} , and the catalysis of CO_2 , as well as numerous other conserved amino acids (see Fig. 1). The protein is 34 to 64 amino acids longer (at the C-terminus) than any previously reported carbonic anhydrase by virtue
20 of the membrane-spanning region also found in HCAIV and an additional approximate 30 amino acids contained in the cytoplasmic side of the cell and apparently missing in other human CA isoforms. In addition, this intracellular domain contains a phosphorylation site recognized by
25 protein kinase C and other kinases, as defined by the motif "Arg-Arg-Lys-Ser" (SEQ ID NO:8 and SEQ ID NO:9) (amino acid residues 1-4 in SEQ ID NO:9 and amino acid residues 299-302 in SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6).

Interestingly, this motif is found only in HCAVIII, and at a functionally significant site, i.e., within the cytosol. A surface cleft essential for enzymatic function present on other carbonic anhydrases is conserved for this protein, suggesting that this protein will also confer enzymatic activity. Five possible N-glycosylation sites are predicted by the primary amino acid sequence and the motif "Asn-Xaa-Ser (Thr)", beginning at amino acid residues -2, 51, 133, 151, and 202 in SEQ ID NO:2, respectively.

HCAVIII is expressed at a much higher level in a non-small cell lung cancer cell line (A549) than in normal lung tissue, other normal tissues, and other tumor cell lines which makes it useful in distinguishing this disease. This is clearly demonstrated in Table 1. Data for this table was obtained as follows. Total cellular RNA was isolated from the indicated actively growing cell lines as described by Chirgwin, et al., "Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease," *Biochemistry* 1979; 18:5294-5299. RNA samples were fractionated over a 1% agarose-formaldehyde gel and transferred to a nylon membrane (Qiagen, Chatsworth, CA) by capillary action. The hybridization probe was generated from a 1 kilobase pair BstXI restriction fragment isolated from pLC56, a plasmid harboring the HCAVIII gene in its initial isolation. This fragment was radiolabeled with ³²P using a PRIME-IT® Random Primer Labeling Kit obtained from Stratagene, La Jolla, CA. A membrane containing RNA derived from healthy human tissue was purchased from Clontech Laboratories, Inc., Palo Alto, CA. RNA blots were hybridized in a standard cocktail containing ³²P-labeled probe at 42°C

overnight then exposed to X-ray film. The same blots were subsequently, upon removal of the probe, rehybridized with a second ³²P-labeled DNA from β -actin to serve as a positive control for integrity of the blotted RNA.

- 5 As shown in Table 1, normal lung tissue does not express the HCAVIII gene in detectable amounts. Other tumor cell lines fail to express, or express only in minor amounts, which will allow easy distinction of non-small cell carcinomas.

TABLE 1. NORTHERN BLOTS USING HCAVIII cDNA AGAINST NORMAL
TISSUES AND TUMOR CELL LINES

<u>TISSUE</u>		<u>mRNA (kB)</u>	<u>INTENSITY</u>
NORMAL TISSUE			
5	heart	nd ¹	-----
	brain	4.5	1X ²
	placenta	4.5	1X
	lung	nd	-----
	liver	nd	-----
10	skeletal muscle	nd	-----
	kidney	4.5	100X
	pancreas	4.5	10X
TUMOR CELL LINE			
15	A549 (lung carcinoma)	3.5	5000X
		5.4	50X
		8.0	25X
		9.0	25X
20	BT20 (breast carcinoma)	nd	-----
	G361 (melanoma)	nd	-----
	HT144 (melanoma)	nd	-----
	U937 (histiocytic lymphoma)	nd	-----
25	KG-1 (myelogenous leukemia)	nd	-----
	¹ nd = none detected ² 1X = at limit of detection		

In one embodiment of the invention, probes are made corresponding to sequences of the cDNA shown in SEQ ID NO:3, which are complimentary to the mRNA for HCAVIII. These probes can be radioactively or non-radioactively labeled in a number of ways well known to the art. The probes can be made of various lengths. Such factors as stringency and GC content may influence the desired probe length for particular applications. The probes correspond to a length of 10-986 nucleotides from SEQ ID NO:3. The labeled probes can then be bound to detect the presence or absence of mRNA encoding the HCAVIII in biopsy material through *in situ* hybridization. The mRNA is expected to be associated with the presence of non-small cell tumors and to be a marker for the precancerous condition as well.

In situ hybridization provides a specificity to the target tissue that is not obtainable in Northern, PCR or other probe-driven technologies. *In situ* hybridization permits localization of signal in mixed-tissue specimens commonly found in most tumors and is compatible with many histologic staining procedures. This technique is comprised of three basic components: first is the preparation of the tissue sample provided by the pathologist to permit successful hybridization to the probe. Second is the preparation of the hybridization probe, typically a RNA complementary to the mRNA of the gene of interest (i.e., antisense RNA). RNA probes are preferred over DNA probes for *in situ* hybridizations mainly because background hybridization of the probe to irrelevant nucleic acids or nonspecific attachment to cell debris or subcellular organelles can be eliminated with RNase treatment post-hybridization. Third is the hybridization and post-hybridization detection. Typically

the RNA transcript probe has been radiolabeled by the incorporation of ^{32}P or ^{35}S nucleotides to permit subsequent detection of the probed specimen by autoradiography or quantitation of silver grains following treatment with autoradiographic emulsion. Nonradioactive detection systems have also been developed. In one example, biotinylated nucleotides can be substituted for the radioactive nucleotide in the RNA probe preparation, permitting visualization of the probed sample by immunocytochemistry-derived techniques. Example 1 describes *in situ* hybridization procedures using RNA probes derived from the HCAVIII gene. Example 2 provides exemplary fluorescent *in situ* (FISH) hybridization procedures.

The cDNA for HCAVIII (SEQ ID NO:3) is currently in an expression vector which is be used to generate the protein in *E. coli*. This expression system described in Example 3 produces HCAVIII to be used as an antigen for the generation of antibodies (Example 4) for use in an ELISA assay to detect shed HCAVIII in body fluids as described in Example 5. The methods for production of antibodies and ELISA type assays are well known in the art. Exemplary methods and components of these procedures have been chosen and developed and are described in Examples 4 and 5.

The expression and purification of foreign proteins in *E. coli* is often problematic. On occasion, the protein is expressed at high levels but is deposited within the cell as an insoluble, denatured form termed an inclusion body. These bodies are often observed when the foreign protein contains a hydrophobic domain, such as found in the membrane spanning segment of HCAVIII. Through

recombinant DNA technology, the DNA sequences encoding the membrane spanning segment of HCAV VIII are deleted. The protein expressed in *E. coli* from this engineered plasmid is now in a soluble and native form within the cell, 5 permitting a rapid and less harsh purification. In addition, the ELISA test to measure HCAV VIII shed into body fluids as described in Example 5 relies on the recombinant protein produced from *E. coli*. Typically, the shed 10 antigen is a membrane-bound receptor that was released from the membrane spanning segment anchoring it to the cell. Consequently, the recombinant HCAV VIII engineered to remove the membrane spanning segment is a more accurate representation of the putative HCAV VIII shed antigen found in specimens and may prove to be the preferred antigen for 15 polyclonal antisera and monoclonal antibody production as described for the development of an ELISA test.

To produce the engineered plasmid, a first plasmid is constructed by cleaving pLC56 with the restriction enzyme Tth111 I, followed by treatment with T₄-DNA polymerase and 20 dGTP, dATP, dTTP and dCTP, and finally with alkaline phosphatase to remove 5'-terminal phosphates. The DNA sample is then purified by phenol/chloroform extraction and ethanol precipitation. The sample is digested with the restriction endonuclease BspE1, then the fragments are 25 resolved by agarose gel electrophoresis to permit the isolation of a 267 base pair fragment. A second plasmid described previously for expression of the HCAV VIII mature protein (SEQ ID NO:4), is cleaved with EcoRI and BspE1 followed by alkaline phosphatase treatment and 30 purification by phenol/chloroform extraction and ethanol precipitation. Two oligonucleotides are synthesized, being 5'-TGAGTCGACG (SEQ ID NO:10) and 5'-AATTCGTCGACTCA

(SEQ ID NO:11), that complement each other and upon annealing, provide a termination codon (TGA) and sequence complementary to EcoRI cleaved DNA. Finally, the two oligonucleotides, the 267 base pair fragment, and the BspEI/EcoRI cleaved plasmid will be combined in a ligation reaction, and the resultant plasmid which contains the truncated DNA sequence (SEQ ID NO:12) is used to transform competent *E. coli*. Upon expression in *E. coli*, the resulting truncated protein (SEQ ID NO:13) is 271 amino acids as determined by SDS polyacrylamide electrophoresis and of a size consistent with other HCA's but lacking the membrane spanning segment and the intracellular domain. A second plasmid encoding a HCAVIII truncated protein (SEQ ID NO:14) lacking the membrane spanning segment and intracellular domain was created as described above, except that restriction enzyme Ple I was substituted for Tth111 I, resulting in a gel purified DNA fragment of 276 base pairs. Upon expression in *E. coli*, the resulting protein is now 274 amino acids (SEQ ID NO:15).

An understanding of protein phosphorylation and its role in the mechanism of cell transformation has been actively pursued, most notably with tyrosine phosphorylation and oncogene activation. The role of serine/threonine protein phosphorylation by a variety of protein kinases including protein kinase C has been studied extensively with respect to signal transduction, but its role in oncogenesis is less clear. To provide a valuable tool to be used in the study of the role of HCAVIII serine phosphorylation in oncogenesis, an altered cDNA can be prepared to code for an altered protein. Changes to amino acids other than "Gly" may be realized by alterations to the oligonucleotide sequence (SEQ ID NO:16)

used to encode the selected residue. Other modifications to alter the serine phosphorylation site would utilize the described technology to modify either both "Arg" residues located within SEQ ID NO:9 or amino acid residues 299 and 300 of SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6. Since "Arg" residues contain a net positive charge, the substituted amino acids would preferably be "Lys" or "His," also positively charged amino acids. An exemplary plasmid is produced in which the "Ser" codon (amino acid residue 4 of SEQ ID NO:9; amino acid residue 302 in SEQ ID NO:2, SEQ ID NO:4 and SEQ ID NO:6), is converted to a "Gly" codon using an *in vitro* mutagenesis technique described in Example 3 and previously recited in Kunkel, Thomas, "Rapid and efficient site-specific mutagenesis without phenotypic selection," *Proc Natl Acad Sci USA* 1985; 82:488-492, and the oligonucleotide 5'-CTTTTTTGATACCCTTCCTTCTGAA (SEQ ID NO:16) (located in SEQ ID NO:1 at the base pairs 1010-1034 with 1022 as the mutagenized base pair). The DNA sequences containing the HCAVIII gene engineered for production of the mature protein and mutagenized codon is released from the mutagenesis vector by BamHI and EcoRI restriction endonucleases and ligated into pGEX4T1 cleaved with the same enzymes, and the resultant plasmid is used to transform competent *E. coli*. The codon mutagenesis is confirmed by DNA sequence analysis, and the protein is expressed and purified from *E. coli* as described in Example 3. The DNA sequence of the altered plasmid as shown in SEQ ID NO:17 differs from the gene encoding the mature protein (SEQ ID NO:3) in that the nucleotide 1022 is changed from "A" to "G", and the protein sequence (SEQ ID NO:18) expressed by the altered plasmid is identical to

the mature protein (SEQ ID NO:4) except that amino acid residue 302 is changed from "Ser" to "Gly."

Another way to detect the presence of increased HCAVIII could be to assay for levels of carbonic anhydrase activity in biopsy materials as described in Example 6. This should be a useful test as HCAVIII, although it is an immunologically unique molecule, contains small but distinct regions which are conserved between previously reported carbonic anhydrase proteins.

In another embodiment of the invention, primers are made complimentary to the HCAVIII cDNA (SEQ ID NO:3) for detecting expression of the gene. PCR amplification of cDNA from lung biopsy cells would indicate the presence of the same non-small cell lung carcinoma.

Due to the non-small cell lung cancer specificity of HCAVIII and the gene encoding the protein, antibodies specific for HCAVIII would also exhibit non-small cell lung cancer specificity which can be employed for diagnostic detection of HCAVIII in body fluids such as serum or urine or HCAVIII containing cells. Targeting of cancer therapeutic drugs to HCAVIII containing cells can also be developed using HCAVIII specific antibodies. The genetic expression of the gene encoding HCAVIII could be modulated by drugs or anti-sense technology resulting in an alteration of the cancer state of the HCAVIII containing cells.

Example 1

In Situ Hybridization using RNA Probes Derived from the HCAVIII Gene

Tissue samples are treated with 4% paraformaldehyde (or equivalent fixative), dehydrated in sequential ethanol solutions of increasing concentrations (e.g., 70%, 95% and 100%) with a final xylene incubation (see *Current Protocols in Molecular Biology*, pp. 14.01-14.3 and *Immunocytochemistry II: IBRO Handbook Series: Methods in the Neurosciences* Vol 14; pp 281-300, incorporated herein by reference). The tissue is embedded in molten paraffin, molded in a casting block and can be stored at room temperature. Tissue slices, typically 8 μ m thick, are prepared with a microtome, dried onto gelatin-treated glass slides and stored at -20°C.

DNA sequences from the HCAVIII gene (SEQ ID NO:3) are subcloned into a plasmid engineered for production of RNA probes. In this example, a 776 bp DNA fragment is released from a pLC56 plasmid following BamHI/AccI digestion, where the BamHI site has been created by in vitro mutagenesis (see *E. coli* expression below). This fragment is ligated into pGEM-2 (Promega Biotec, Madison, WI) that was cleaved with BamHI and AccI and transformed into competent *E. coli*. This constructed plasmid contains the T7 RNA polymerase promoter downstream of the AccI restriction site and hence can drive transcription of the antisense HCAVIII sequences defined by the BamHI/AccI fragment. Following linearization of the subsequent plasmid with BamHI, an in vitro transcription reaction composed of transcription buffer (40 mM Tris-HCl, pH 7.5, 6 mM MgCl₂, 2 mM spermidine, 10 mM NaCl, 10 mM dithiothreitol, 1 U/ μ l ribonuclease inhibitor), linearized plasmid, 10 mM GTP, 10 mM ATP, 10 mM CTP, 100 μ Ci of (³⁵S)UTP, and T7 RNA polymerase is incubated at 37°C. Multiple RNA copies of the gene are produced that then are

used as a hybridization probe. The reaction is terminated by the addition of DNAase, and the synthesized RNA is recovered from unincorporated nucleotides by phenol/chloroform extraction and sequential ethanol precipitations in the presence of 2.5 M ammonium acetate.

The slides containing fixed, sectioned tissues are rehydrated in decreasing concentration of ethanol (100%, 70% and 50%), followed by sequential treatments with 0.2 N HCl, 2X SSC (where 20X SSC is 3 M NaCl and 0.3 M sodium citrate) at 70°C to deparaffinate the sample, phosphate buffered saline (PBS), fixation in 4% paraformaldehyde and PBS wash. The slides are blocked to prevent nonspecific binding by the sequential additions of PBS/10mM dithiothreitol (45°C), 10 mM dithiothreitol/0.19% iodoacetamide/0.12% N-ethylmaleimide and PBS wash. The slides are equilibrated in 0.1M triethylamine, pH 8.0, followed by treatment in 0.1M triethylamine/0.25% acetic anhydride and 0.1 M triethylamine/0.5% acetic anhydride and washed in 2X SSC. The slides are then dehydrated in increasing concentrations of ethanol (50%, 70% and 100%) and stored at -80°C.

A hybridization mix is prepared by combining 50% deionized formamide, 0.3 M NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1X Denhardt's solution (0.02% Ficoll 400, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin (BSA)), 500 µg/ml yeast tRNA, 500 µg/ml poly(A), 50 mM dithiothreitol, 10% polyethyleneglycol 6000 and the ³⁵S-labeled RNA probe. This solution is placed on the fixed, blocked tissue slides which are then incubated at 45°C in a moist chamber for 0.5 to 3 hours. The slides are washed to remove unbound probe in 50% formamide, 2X SSC, 20 mM 2-mercaptoethanol (55°C), followed by 50% formamide, 2X SSC,

20 mM 2-mercaptoethanol and 0.5% Triton-X 100 (50°C) and finally in 2X SSC/20 mM 2-mercaptoethanol (room temperature). The slides are treated with 10 mM Tris-HCl, pH 8.0/0.3 M NaCl/40 µg/ml RNase A/2 µg/ml RNase T1 (37°C) to reduce levels of unbound RNA probe. Following RNase treatment, the slides are washed in formamide/SSC buffers at 50°C, room temperature and then dehydrated in increasing ethanol concentrations containing 0.3 M ammonium acetate, and one final 100% ethanol wash. The slides are then exposed to X-ray film followed by emulsion autoradiography to detect silver grains.

Test tissue samples are compared to matched controls derived from normal lung tissue. Evidence of elevated transcription of the HCAVIII gene in test tissue compared to normal tissue, as determined by autoradiography (X-ray film) or alternatively by the quantitation of silver grains following emulsion autoradiography would provide evidence of a positive diagnosis for lung cancer.

Example 2

Fluorescent In Situ Hybridization (FISH) Using DNA Probes Derived from the HCAVIII Gene

A genomic clone to the HCAVIII gene (SEQ ID NO:1) is isolated using a PCR primer pair which have been identified from the pLC56 cDNA sequence. This primer pair is located in putative exon 6 of the pLC56 gene, and they are identified as Probe Exon 6A (5'-ACATTGAAGAGCTGCTTCCGG-3'; SEQ ID NO:19) and Probe Exon 6B (5'-AATTTGCACGGGGTTTCGG-3'; SEQ ID NO:20). The genomic clone of HCAVIII is then identified as a PCR product of about 119 bp using this primer pair from the designated genomic clone. This result is confirmed by Southern blotting and

DNA sequence analysis. A sequence of 1363 bp derived from the HCAVIII genomic clone is reported in SEQ ID NO:21. This sequence is located directly before the HCAVIII cDNA and constitutes the putative promoter of this gene and likely contains transcription regulatory elements directly implicated in HCAVIII expression.

The DNA probe comprising the genomic clone of HCAVIII plus flanking sequences is labeled in a random primer reaction with digoxigenin-11-dUTP (Boehringer Mannheim Biochemicals, Indianapolis, IN) by combining the DNA with dNTP(-TTP, final 0.05 mM), digoxigenin-11-dUTP/dTTP (0.0125 mM and 0.0375 mM, final), 10 mM 2-mercaptoethanol, 50 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 20 U of DNA polymerase I and 1 ng/ml DNAase. The reaction is incubated at 15°C for two hours, and then terminated by adding EDTA to a final concentration of 10 mM. The labeled DNA probe is further purified by gel filtration chromatography. It is apparent to those skilled in the art that other suitable substrates such as biotin-11-dUTP can be substituted for digoxigenin-11-dUTP in the procedure above.

A hybridization mix is prepared by combining 50% deionized formamide, 0.3 M NaCl, 10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 1X Denhardt's solution (0.02% Ficoll 400, 0.02% polyvinylpyrrolidone, and 0.02% bovine serum albumin), 500 µg/ml yeast tRNA, 500 µg/ml poly(A), 50 mM dithiothreitol, 10% polyethyleneglycol 6000, and the labeled DNA probe.

Single cell suspensions of tissue biopsy material or normal tissue are fixed in methanol/glacial acetic acid (3:1 vol/vol) and dropped onto microscope slides. (Aanastasi, et al., "Detection of Trisomy 12 in chronic lymphocytic leukemia by fluorescence in situ hybridization

to interphase cells: a simple and sensitive method," *Blood* 1992; 77:2456-2462). After the slides are heated for 1-2 hours at 60°C, the hybridization mix is applied to the slides which are then incubated at 45°C in a moist chamber for 0.5-3 hours. After incubation, the slides are washed three times with a solution comprising 50% formamide and 2X SSC at 42°C, washed twice in 2X SSC at 42°C, and finally washed in 4X SSC at room temperature. The slide is blocked with a solution of 4X SSC and 1% BSA, and then washed with a solution of 4X SSC and 1% Triton X-100.

The hybridized digoxigenin-labeled probe is detected by adding a mixture of sheep anti-digoxigenin antibody (Boehringer Mannheim) diluted in 0.1 M sodium phosphate, pH 8.0, 5% nonfat dry milk, and 0.02% sodium azide, followed by the addition of fluorescein-conjugated rabbit anti-sheep IG for detection. The slides are then washed in PBS, mounted in Vectashield (Vector Laboratories, Inc., Burlingame, CA), and viewed by fluorescent microscopy.

Hybridization signals are enumerated in tumor derived tissue and then compared to normal tissue. Normal tissue displays two distinct hybridization signal characteristics of a diploid state. Enumeration over the rate of two hybridization signals/cell is considered significant.

Example 3

Expression of HCAVIII

Expression of foreign proteins is often performed in *E. coli* when an immunogen or large amounts of protein are desired, as in the development of a diagnostic kit. A preferred system for *E. coli* expression has been described (Smith, et al., "Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione-

s-transferase," Gene 1988; 67:31-40) whereby glutathione transferase is expressed with amino acids representing the cloned protein of interest attached to the carboxyl-terminus. The fusion protein can then be purified via
5 affinity chromatography and the protein of interest fused to glutathione transferase released by digestion with the protease thrombin or alternatively the fusion protein is released intact from the affinity column by competing levels of free glutathione.

10 To express the HCAVIII protein (SEQ ID NO:4) of this invention in *E. coli* using the above described technology, an expression plasmid was produced fused to the glutathione transferase gene in frame with the HCAVIII gene (SEQ ID NO:1) to produce a fusion protein. The
15 fusion gene/expression plasmid was assembled from nucleic acids derived from the following sources. First, the expression plasmid pGEX4T1 (Pharmacia, Piscataway, NJ) was cleaved in the polycloning region with the restriction endonucleases BamHI and EcoRI to permit insertion of the
20 HCAVIII gene. Second, an oligonucleotide was synthesized, being 5'-GTCCACTTGGATCCGTTCACTGG-3' (SEQ ID NO:22). Using the in vitro mutagenesis procedure described by Kunkel (Proc Natl Acad Sci USA 1985; 82:488-492) and the above oligonucleotide, a BamHI restriction site was created
25 without altering the amino acid codons of the original protein. In addition the created BamHI site was situated in correct reading frame and proximity to the predicted cleavage site separating the signal peptide from the mature protein. The DNA sequences encoding the mature
30 protein were released from the mutagenesis vector as a BamHI/EcoRI fragment, where the EcoRI site originates from a polycloning region of the DNA sequencing vector pUC19

found downstream of the HCAVIII gene. The DNA fragments described above comprised of pGEX4T-1 cleaved at BamHI and EcoRI and the HCAVIII gene released as a BamHI/EcoRI fragment was combined in a mixture composed of 1X T₄ ligase buffer (50 mM Tris-HCl, 10 mM MgCl₂, 20 mM dithiothreitol, 1 mM ATP, 50 µg/ml BSA, final pH 7.5) and T₄ DNA ligase (New England Biolabs, Beverly, MA). The ligated DNA was used to transform a suitable strain of *E. coli* such as XL-1 Blue (Stratagene). The recovered plasmid is sequenced to confirm the expected DNA sequence. Protein expression is induced in *E. coli* with the chemical isopropyl β-thiogalactoside, and the fusion protein is released by cell lysis, followed by denaturation and resolubilization of the fusion protein with 8 M urea/ 20 mM Tris.Cl (pH 8.5)/10 mM dithiothreitol, dialysis and protein renaturation, and finally binding to an affinity column composed of glutathione-agarose (Sigma, St. Louis, MO) and cleavage with thrombin to release the HCAVIII protein. The resulting protein is suitable as an immunogen for polyclonal or monoclonal antibody production and for usage in an ELISA kit as a internal standard and positive control. Carbonic anhydrase enzyme activity (as described in Example 6) was measured for *E.coli*-derived HCAVIII and HCAVIII-truncated form (SEQ ID NO:15) and compared to commercially obtained human carbonic anhydrase II (Sigma, St. Louis, Mo.). The activity, as reported in Enzyme Unit (U)/mg, for human carbonic anhydrase II was 3571 U/mg, for HCAVIII was 274 U/mg and HCAVIII truncated form was 2632 U/mg. These results indicated an enzymatically active and renaturable HCAVIII derived from *E.coli* of comparable enzymatic activity to human carbonic anhydrase II was obtained.

The length of the resulting protein can be varied by altering the length of SEQ ID NO:1 prior to insertion into the expression plasmid, or by cleavage of amino acids from the protein resulting in the above example. Structure/

5 function studies of other HCA's suggest modifications (as defined by deletions at the N-terminal and C-terminal) more extensive than disclosed in SEQ ID NO:12 would still permit the production and use of a protein as an immunogen or standard, these deletions being a protein defined by

10 about amino acid residue 3 to amino acid residue 259 in SEQ ID NO:12. Using existing technology one could synthesize a peptide of approximately 10 to 40 amino acids in length that comprises a structural domain of HCAVIII. This synthesized peptide, coupled to a carrier protein,

15 could be used for generating polyclonal antisera specific for native HCAVIII.

Example 4

Production of Antibodies to HCAVIII

The production of polyclonal antisera is described in

20 great detail in Harlow, et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratories, New York, 1988 incorporated herein by reference. The HCAVIII protein (SEQ ID NO:4) in the presence of an adjuvant is injected into rabbits with a series of booster shots as a

25 prescribed schedule optimal for high titers of antibody in serum. A total of seven biweekly bleeds were obtained from two rabbits immunized with HCAVIII truncated protein (SEQ ID NO:15). The resulting anti-HCAVIII serum titer was compared to preimmune sera of the same rabbits and

30 determined to be 1000 to 2000-fold greater, hence suitable as a reagent for indirect ELISA (Example 5). Rabbit

antibody was partially purified by precipitation with ammonium sulfate (50%, final) followed by dialysis and fractionation by preparative DEAE-HPLC.

5 An extensive description for producing monoclonal antibodies derived from the spleen B cells of an immunized mouse and a immortalized myeloma cell is found in the above reference for polyclonal antisera production. Mice are immunized with either the purified HCAVIII protein or a glutathione/HCAVIII fusion protein. Following cell
10 fusion, selection for hybrid cells and subcloning, hybridomas are screened for a positive antibody against whole A549 cells or purified HCAVIII protein using an indirect ELISA assay as described for the ELISA kit (see Example 5).

15

Example 5

ELISA Assay of Shed HCAVIII

 An indirect ELISA screening assay for HCAVIII protein (SEQ ID NO:4) has been designed to detect and monitor the HCAVIII protein in body fluids including but not limited
20 to serum and other biological fluids such as sputum or bronchial effluxion at effective levels necessary for sensitive but accurate determinations. It is intended to aid in the early diagnosis of non-small cell lung cancer, for which there currently is no effective treatment. An
25 early-detection, accurate, non-invasive assay for non-small cell lung cancer would be of great benefit in the management of this disease.

 The immunochemicals used in this procedure were rabbit anti-human HCAVIII antibody (purified IgG, IgM)
30 produced according to the procedure given in Example 4, mouse anti-human HCAVIII (monoclonal) also produced

according to the procedure given in Example 4, and goat anti-Rabbit IgG/peroxidase conjugate. The HCAVIII protein standard and internal positive control were produced as described in Example 3 for expression in *E. coli*.

5 Substrate components include 1 M H₂SO₄ stored at room temperature and 3',5,5'-tetramethylbenzidine (TMB) (Sigma Chemical Co.) used as a peroxidase substrate and stored at room temperature in the dark to prevent exposure to light.

10 Several buffers, diluents, and blocking agents were used in the procedure. Note that no sodium azide preservative was used in any of the buffers. This was done to avoid any possible interference from the azide with the peroxidase conjugate.

15 Phosphate buffered saline (PBS) was prepared by adding 32.0 g sodium chloride, 0.8 g potassium phosphate, monobasic, 0.8 g potassium chloride, and 4.6 g sodium phosphate, dibasic, anhydrous, to 3.2 L deionized water and mixing to dissolve. After bringing the solution to 4 L with deionized water and mixing, the pH was about 7.2.
20 The buffer can be stored at 4°C for a maximum of 3 weeks.

25 Two bovine serum albumin solutions (BSA) were utilized as diluents. A 1% BSA solution in PBS, utilized as the second antibody/conjugate diluent, was prepared by adding 1 g BSA (bovine albumin, Fraction V, Sigma Chemical Co.) to 80 ml of PBS, allowing it to stand as it slowly goes into solution, adding PBS to a final volume of 100 ml, and then mixing. This diluent can be stored at 4°C for a maximum of 2 weeks; however if the solution becomes
30 turbid, it should be discarded. As a diluent for the standards and samples, a 0.025% BSA solution in PBS was prepared fresh for each assay by diluting the 1% BSA diluent with PBS 1:40 (vol/vol).

A borate blocking buffer (0.17 M H_3BO_3 , 0.12 M NaCl, 0.05% Tween 20, 1mM EDTA and 0.25% BSA was also used.

The substrate buffer was phosphate-citrate/sodium per borate (Sigma, St. Louis, Mo.).

5 All assays were performed in Immulon IV plates (Dynatech, Chantilly, VA #011-010-6301). The assay plates were coated with a monoclonal antibody against HCAVIII by adding 50 ul of a 10 ug/ml solution of antibody in PBS to each well of Immulon IV plates. The plates were covered
10 and incubated overnight at room temperature. The antibody solution was removed and the wells rinsed three times with deionized water. Three-hundred microliters (300 ul) of the borate blocking buffer was added to each well and incubated at room temperature for thirty minutes. The
15 buffer was removed, the wells rinsed three times with deionized water, and the plates air dried. The plates were then wrapped and stored at 4°C.

The standard E.coli-derived HCAVIII truncated protein (SEQ ID NO:15), was diluted to 32 ng/ml in PBS/0.025% BSA
20 and two-fold serial dilutions were made in same. The samples were also diluted in PBS/0.025% BSA and 50 ul of standard or sample was applied to each well. The plates were incubated overnight, covered, at room temperature.

The standard and sample solutions were removed from
25 the wells and the wells were rinsed three times with deionized water. Three-hundred microliters (300 ul) borate blocking buffer was added to each well and incubated at room temperature for thirty minutes. The plates were rinsed again with deionized water and tapped
30 (inverted) on paper towels to remove excess water. The second antibody rabbit antisera to HCAVIII truncated protein (SEQ ID NO:15), was diluted to 1 ug/ml in PBS/1%

BSA and 50 ul was added to each well. The plates were covered and incubated at room temperature two hours.

The antibody solution was removed from the wells which were then rinsed with deionized water three times.

5 They were then blocked for ten minutes at room temperature with borate blocking buffer, rinsed again with deionized water three times, and tapped on paper towels. The antibody conjugate, goat F(ab')₂ x rabbit IgG & IgL-HPRO (Tago, Camarillo, CA.) was diluted 1:16,000 in PBS/1%BSA
10 and 50 ul was added to each well. The plates were covered and incubated at room temperature two hours.

The antibody conjugate solution was removed from the wells and they were rinsed with deionized water three times, blocked with three-hundred ul borate buffer at room
15 temperature then minutes, rinsed three times with deionized water, and tapped on paper towels. The substrate was prepared no more than fifteen minutes before use by dissolving one capsule of phosphate-citrate/sodium perborate (Sigma, St. Louis, Mo.) in 100 ml water. For
20 each plate, one tablet of TMB was added to 10 ml of the phosphate-citrate/sodium perborate buffer and syringe filtered. One-hundred ul was added to each well and the plates were covered and incubated at room temperature in the dark for one hour. The reaction was stopped by adding
25 50 ul of 1M H₂SO₄ to each well. The plates were read on a Molecular Devices microplate reader at 450nm. Under these conditions, a linear response was obtained from 0.5 to 32 ng/ml using HCAVIII truncated protein as a standard, with the assay sensitivity at 0.5 ng/ml. No cross-reaction was
30 observed against HCAII, an abundant carbonic anhydrase in human serum.

Example 6**Carbonic Anhydrase (CA) Activity of Biopsy Tissue**

Ice cold solutions of ITB (20 mM imidazole, 5 mM Tris, and 0.4 mM para-nitrophenol, pH 9.4-9.9) and Buffer A (25 mM triethanolamine, 59 mM H₂SO₄, and 1 mM benzamidine HCl) are prepared.

A homogenate is prepared by scraping with a cell scraper into 1-2 ml of Buffer A a monolayer of tissue cells cultured from a tissue sample taken from a biopsy. A portion of the sample is then boiled to inactivate CA.

A tube is placed in an ice water bath. For the macroassay, a 10 x 75 mm glass tubes and rubber stopper with 16 gauge and 18 gauge needle ports is used; for the microassay, a 6 x 50 mm glass tubes and rubber stopper with 18 gauge needle port and 20 gauge needle with attached PE90 tubing. The sample is added and along with ice cold water to a final volume of 500 µl for macroassay or 50 µl for microassay. 500 µl (macro) or 50 µl (micro) ice cold water is used for a water control. 10 µl antifoam (A. H. Thomas, Philadelphia, PA) is added to the tube which is then incubated in ice water for 0.5 to 3 minutes.

The tube is capped with a stopper and CO₂ at 150 ml/min (macro) or 100 ml/min (micro) is bubbled through the smaller needle port for 30 sec.

50 µl (macro) or 50 µl (micro) of the ITB solution is rapidly added through the larger needle port with a cold Hamilton syringe. The sample becomes yellow.

Using a timer or stopwatch, the time at which the solution in the tube becomes colorless is measured and recorded. The tube may be momentarily removed from the bath and held in front of a white background to determine

the color change. Comparison to a previously acidified sample may be used.

The procedure is repeated with the boiled sample. The volume of sample that corresponds to approximately one enzyme unit is determined using the formula below.

5

Volume (1EU) = V_{EU} = volume used x log2/log (boiled time/activated time) One enzyme unit is the activity that halves the boiled control time.

The assay is repeated 1-3 times with the sample and boiled sample, using the adjusted volume of sample.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Cytoclonal Pharmaceuticals, Inc.
(B) STREET: 9000 Harry Hines Blvd, Suite 330
(C) CITY: Dallas
(D) STATE: Texas
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 75235
(G) TELEPHONE: (214) 353-2923
(H) TELEFAX: (214) 350-9514
(I) TELEX:

(ii) TITLE OF INVENTION: Lung Cancer Marker

(iii) NUMBER OF SEQUENCES: 22

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: RICHARDS, MEDLOCK & ANDREWS
(B) STREET: 1201 Elm Street, Suite 4500
(C) CITY: Dallas
(D) STATE: TX
(E) COUNTRY: US
(F) ZIP: 75270-2197

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: John A. Harre
(B) REGISTRATION NUMBER: 37,345
(C) REFERENCE/DOCKET NUMBER: B35792CIPPCT

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 214-939-4500
(B) TELEFAX: 214-939-4600

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1104 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: both
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: CDS
(B) LOCATION: 32..1093

(ix) FEATURE:

(A) NAME/KEY: mat_peptide
(B) LOCATION: 119..1093

(ix) FEATURE:

(A) NAME/KEY: misc_feature
(B) LOCATION: 1013..1024
(D) OTHER INFORMATION: /note= "phosphorylation site
recognized by protein kinase C and other kina..."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```

GCCCCGCGCCC GCCCCGCAGG AGCCCCGCGAA G ATG CCC CGG CGC AGC CTG CAC      52
Met Pro Arg Arg Ser Leu His
-29 -25

GCG GCG GCC GTG CTC CTG CTG GTG ATC TTA AAG GAA CAG CCT TCC AGC      100
Ala Ala Ala Val Leu Leu Leu Val Ile Leu Lys Glu Gln Pro Ser Ser
-20 -15 -10

CCG GCC CCA GTG AAC GGT TCC AAG TGG ACT TAT TTT GGT CCT GAT GGG      148
Pro Ala Pro Val Asn Gly Ser Lys Trp Thr Tyr Phe Gly Pro Asp Gly
-5 1 5 10

GAG AAT AGC TGG TCC AAG AAG TAC CCG TCG TGT GGG GGC CTG CTG CAG      196
Glu Asn Ser Trp Ser Lys Lys Tyr Pro Ser Cys Gly Gly Leu Leu Gln
15 20 25

TCC CCC ATA GAC CTG CAC AGT GAC ATC CTC CAG TAT GAC GCC AGC CTC      244
Ser Pro Ile Asp Leu His Ser Asp Ile Leu Gln Tyr Asp Ala Ser Leu
30 35 40

ACG CCC CTC GAG TTC CAA GGC TAC AAT CTG TCT GCC AAC AAG CAG TTT      292
Thr Pro Leu Glu Phe Gln Gly Tyr Asn Leu Ser Ala Asn Lys Gln Phe
45 50 55

CTC CTG ACC AAC AAT GGC CAT TCA GTG AAG CTG AAC CTG CCC TCG GAC      340
Leu Leu Thr Asn Asn Gly His Ser Val Lys Leu Asn Leu Pro Ser Asp
60 65 70

ATG CAC ATC CAG GGC CTC CAG TCT CGC TAC AGT GCC ACG CAG CTG CAC      388
Met His Ile Gln Gly Leu Gln Ser Arg Tyr Ser Ala Thr Gln Leu His
75 80 85 90

CTG CAC TGG GGG AAC CCG AAT GAC CCG CAC GGC TCT GAG CAC ACC GTC      436
Leu His Trp Gly Asn Pro Asn Asp Pro His Gly Ser Glu His Thr Val
95 100 105

AGC GGA CAG CAC TTC GCC GCC GAG CTG CAC ATT GTC CAT TAT AAC TCA      484
Ser Gly Gln His Phe Ala Ala Glu Leu His Ile Val His Tyr Asn Ser
110 115 120

```

42

GAC CTT TAT CCT GAC GCC AGC ACT GCC AGC AAC AAG TCA GAA GGC CTC Asp Leu Tyr Pro Asp Ala Ser Thr Ala Ser Asn Lys Ser Glu Gly Leu 125 130 135	532
GCT GTC CTG GCT GTT CTC ATT GAG ATG GGC TCC TTC AAT CCG TCC TAT Ala Val Leu Ala Val Leu Ile Glu Met Gly Ser Phe Asn Pro Ser Tyr 140 145 150	580
GAC AAG ATC TTC AGT CAC CTT CAA CAT GTA AAG TAC AAA GGC CAG GAA Asp Lys Ile Phe Ser His Leu Gln His Val Lys Tyr Lys Gly Gln Glu 155 160 165 170	628
GCA TTC GTC CCG GGA TTC AAC ATT GAA GAG CTG CTT CCG GAG AGG ACC Ala Phe Val Pro Gly Phe Asn Ile Glu Glu Leu Leu Pro Glu Arg Thr 175 180 185	676
GCT GAA TAT TAC CGC TAC CGG GGG TCC CTG ACC ACA CCC CCT TGC AAC Ala Glu Tyr Tyr Arg Tyr Arg Gly Ser Leu Thr Thr Pro Pro Cys Asn 190 195 200	724
CCC ACT GTG CTC TGG ACA GTT TTC CGA AAC CCC GTG CAA ATT TCC CAG Pro Thr Val Leu Trp Thr Val Phe Arg Asn Pro Val Gln Ile Ser Gln 205 210 215	772
GAG CAG CTG CTG GCT TTG GAG ACA GCC CTG TAC TGC ACA CAC ATG GAC Glu Gln Leu Leu Ala Leu Glu Thr Ala Leu Tyr Cys Thr His Met Asp 220 225 230	820
GAC CCT TCC CCC AGA GAA ATG ATC AAC AAC TTC CGG CAG GTC CAG AAG Asp Pro Ser Pro Arg Glu Met Ile Asn Asn Phe Arg Gln Val Gln Lys 235 240 245 250	868
TTC GAT GAG AGG CTG GTA TAC ACC TCC TTC TCC CAA GTG CAA GTC TGT Phe Asp Glu Arg Leu Val Tyr Thr Ser Phe Ser Gln Val Gln Val Cys 255 260 265	916
ACT GCG GCA GGA CTG AGT CTG GGC ATC ATC CTC TCA CTG GCC CTG GCT Thr Ala Ala Gly Leu Ser Leu Gly Ile Ile Leu Ser Leu Ala Leu Ala 270 275 280	964
GGC ATT CTT GGC ATC TGT ATT GTG GTG GTG GTG TCC ATT TGG CTT TTC Gly Ile Leu Gly Ile Cys Ile Val Val Val Val Ser Ile Trp Leu Phe 285 290 295	1012
AGA AGG AAG AGT ATC AAA AAA GGT GAT AAC AAG GGA GTC ATT TAC AAG Arg Arg Lys Ser Ile Lys Lys Gly Asp Asn Lys Gly Val Ile Tyr Lys 300 305 310	1060
CCA GCC ACC AAG ATG GAG ACT GAG GCC CAC GCT TGAGGTCCCC G Pro Ala Thr Lys Met Glu Thr Glu Ala His Ala 315 320 325	1104

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 354 amino acids

(B) TYPE: amino acid

43

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Pro Arg Arg Ser Leu His Ala Ala Ala Val Leu Leu Leu Val Ile
-29          -25          -20          -15

Leu Lys Glu Gln Pro Ser Ser Pro Ala Pro Val Asn Gly Ser Lys Trp
          -10          -5          1

Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys Lys Tyr Pro
  5          10          15

Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His Ser Asp Ile
  20          25          30          35

Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln Gly Tyr Asn
          40          45          50

Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly His Ser Val
          55          60          65

Lys Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu Gln Ser Arg
  70          75          80

Tyr Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro Asn Asp Pro
  85          90          95

His Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala Ala Glu Leu
100          105          110          115

His Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala Ser Thr Ala
          120          125          130

Ser Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu Ile Glu Met
          135          140          145

Gly Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His Leu Gln His
          150          155          160

Val Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe Asn Ile Glu
          165          170          175

Glu Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr Arg Gly Ser
180          185          190          195

Leu Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr Val Phe Arg
          200          205          210

Asn Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu Glu Thr Ala
          215          220          225

Leu Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu Met Ile Asn
          230          235          240

```

44

Asn Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val Tyr Thr Ser
 245 250 255
 Phe Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu Ser Leu Gly Ile
 260 265 270 275
 Ile Leu Ser Leu Ala Leu Ala Gly Ile Leu Gly Ile Cys Ile Val Val
 280 285 290
 Val Val Ser Ile Trp Leu Phe Arg Arg Lys Ser Ile Lys Lys Gly Asp
 295 300 305
 Asn Lys Gly Val Ile Tyr Lys Pro Ala Thr Lys Met Glu Thr Glu Ala
 310 315 320
 His Ala
 325

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 986 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..975

(ix) FEATURE:

- (A) NAME/KEY: misc_feature
- (B) LOCATION: 895..906
- (D) OTHER INFORMATION: /note= "phosphorylation site recognized by protein C kinase and other kina..."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TCC AAG TGG ACT TAT TTT GGT CCT GAT GGG GAG AAT AGC TGG TCC AAG	48
Ser Lys Trp Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys	
1 5 10 15	
AAG TAC CCG TCG TGT GGG GGC CTG CTG CAG TCC CCC ATA GAC CTG CAC	96
Lys Tyr Pro Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His	
20 25 30	
AGT GAC ATC CTC CAG TAT GAC GCC AGC CTC ACG CCC CTC GAG TTC CAA	144
Ser Asp Ile Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln	
35 40 45	
GGC TAC AAT CTG TCT GCC AAC AAG CAG TTT CTC CTG ACC AAC AAT GGC	192
Gly Tyr Asn Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly	
50 55 60	

45

CAT TCA GTG AAG CTG AAC CTG CCC TCG GAC ATG CAC ATC CAG GGC CTC	240
His Ser Val Lys Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu	
65 70 75 80	
CAG TCT CGC TAC AGT GCC ACG CAG CTG CAC CTG CAC TGG GGG AAC CCG	288
Gln Ser Arg Tyr Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro	
85 90 95	
AAT GAC CCG CAC GGC TCT GAG CAC ACC GTC AGC GGA CAG CAC TTC GCC	336
Asn Asp Pro His Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala	
100 105 110	
GCC GAG CTG CAC ATT GTC CAT TAT AAC TCA GAC CTT TAT CCT GAC GCC	384
Ala Glu Leu His Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala	
115 120 125	
AGC ACT GCC AGC AAC AAG TCA GAA GGC CTC GCT GTC CTG GCT GTT CTC	432
Ser Thr Ala Ser Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu	
130 135 140	
ATT GAG ATG GGC TCC TTC AAT CCG TCC TAT GAC AAG ATC TTC AGT CAC	480
Ile Glu Met Gly Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His	
145 150 155 160	
CTT CAA CAT GTA AAG TAC AAA GGC CAG GAA GCA TTC GTC CCG GGA TTC	528
Leu Gln His Val Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe	
165 170 175	
AAC ATT GAA GAG CTG CTT CCG GAG AGG ACC GCT GAA TAT TAC CGC TAC	576
Asn Ile Glu Glu Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr	
180 185 190	
CGG GGG TCC CTG ACC ACA CCC CCT TGC AAC CCC ACT GTG CTC TGG ACA	624
Arg Gly Ser Leu Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr	
195 200 205	
GTT TTC CGA AAC CCC GTG CAA ATT TCC CAG GAG CAG CTG CTG GCT TTG	672
Val Phe Arg Asn Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu	
210 215 220	
GAG ACA GCC CTG TAC TGC ACA CAC ATG GAC GAC CCT TCC CCC AGA GAA	720
Glu Thr Ala Leu Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu	
225 230 235 240	
ATG ATC AAC AAC TTC CGG CAG GTC CAG AAG TTC GAT GAG AGG CTG GTA	768
Met Ile Asn Asn Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val	
245 250 255	
TAC ACC TCC TTC TCC CAA GTG CAA GTC TGT ACT GCG GCA GGA CTG AGT	816
Tyr Thr Ser Phe Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu Ser	
260 265 270	
CTG GGC ATC ATC CTC TCA CTG GCC CTG GCT GGC ATT CTT GGC ATC TGT	864
Leu Gly Ile Ile Leu Ser Leu Ala Leu Ala Gly Ile Leu Gly Ile Cys	
275 280 285	

46

ATT GTG GTG GTG GTG TCC ATT TGG CTT TTC AGA AGG AAG AGT ATC AAA	912
Ile Val Val Val Val Ser Ile Trp Leu Phe Arg Arg Lys Ser Ile Lys	
290 295 300	
AAA GGT GAT AAC AAG GGA GTC ATT TAC AAG CCA GCC ACC AAG ATG GAG	960
Lys Gly Asp Asn Lys Gly Val Ile Tyr Lys Pro Ala Thr Lys Met Glu	
305 310 315 320	
ACT GAG GCC CAC GCT TGAGGTCCCC G	986
Thr Glu Ala His Ala	
325	

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 325 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Ser Lys Trp Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys	
1 5 10 15	
Lys Tyr Pro Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His	
20 25 30	
Ser Asp Ile Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln	
35 40 45	
Gly Tyr Asn Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly	
50 55 60	
His Ser Val Lys Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu	
65 70 75 80	
Gln Ser Arg Tyr Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro	
85 90 95	
Asn Asp Pro His Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala	
100 105 110	
Ala Glu Leu His Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala	
115 120 125	
Ser Thr Ala Ser Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu	
130 135 140	
Ile Glu Met Gly Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His	
145 150 155 160	
Leu Gln His Val Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe	
165 170 175	

47

Asn Ile Glu Glu Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr
 180 185 190
 Arg Gly Ser Leu Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr
 195 200 205
 Val Phe Arg Asn Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu
 210 215 220
 Glu Thr Ala Leu Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu
 225 230 235 240
 Met Ile Asn Asn Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val
 245 250 255
 Tyr Thr Ser Phe Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu Ser
 260 265 270
 Leu Gly Ile Ile Leu Ser Leu Ala Leu Ala Gly Ile Leu Gly Ile Cys
 275 280 285
 Ile Val Val Val Val Ser Ile Trp Leu Phe Arg Arg Lys Ser Ile Lys
 290 295 300
 Lys Gly Asp Asn Lys Gly Val Ile Tyr Lys Pro Ala Thr Lys Met Glu
 305 310 315 320
 Thr Glu Ala His Ala
 325

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2134 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 116..1177

(ix) FEATURE:

- (A) NAME/KEY: mat_peptide
- (B) LOCATION: 203..1177

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GTACTCGCCA CGGCACCCAG GCTGCGCGCA CGCGGTCCCG GTGTGCAGCT GGAGAGCGAG 60
 CGGCCACCGG GAGCCCCCGG CACAGCCCGC GCCCGCCCCG CAGGAGCCCG CGAAG ATG 118
 Met
 -29
 CCC CGG CGC AGC CTG CAC GCG GCG GCC GTG CTC CTG CTG GTG ATC TTA 166

Pro Arg Arg Ser Leu His Ala Ala Ala Val Leu Leu Leu Val Ile Leu	
-25 -20 -15	
AAG GAA CAG CCT TCC AGC CCG GCC CCA GTG AAC GGT TCC AAG TGG ACT	214
Lys Glu Gln Pro Ser Ser Pro Ala Pro Val Asn Gly Ser Lys Trp Thr	
-10 -5 1	
TAT TTT GGT CCT GAT GGG GAG AAT AGC TGG TCC AAG AAG TAC CCG TCG	262
Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys Lys Tyr Pro Ser	
5 10 15 20	
TGT GGG GGC CTG CTG CAG TCC CCC ATA GAC CTG CAC AGT GAC ATC CTC	310
Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His Ser Asp Ile Leu	
25 30 35	
CAG TAT GAC GCC AGC CTC ACG CCC CTC GAG TTC CAA GGC TAC AAT CTG	358
Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln Gly Tyr Asn Leu	
40 45 50	
TCT GCC AAC AAG CAG TTT CTC CTG ACC AAC AAT GGC CAT TCA GTG AAG	406
Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly His Ser Val Lys	
55 60 65	
CTG AAC CTG CCC TCG GAC ATG CAC ATC CAG GGC CTC CAG TCT CGC TAC	454
Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu Gln Ser Arg Tyr	
70 75 80	
AGT GCC ACG CAG CTG CAC CTG CAC TGG GGG AAC CCG AAT GAC CCG CAC	502
Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro Asn Asp Pro His	
85 90 95 100	
GGC TCT GAG CAC ACC GTC AGC GGA CAG CAC TTC GCC GCC GAG CTG CAC	550
Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala Ala Glu Leu His	
105 110 115	
ATT GTC CAT TAT AAC TCA GAC CTT TAT CCT GAC GCC AGC ACT GCC AGC	598
Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala Ser Thr Ala Ser	
120 125 130	
AAC AAG TCA GAA GGC CTC GCT GTC CTG GCT GTT CTC ATT GAG ATG GGC	646
Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu Ile Glu Met Gly	
135 140 145	
TCC TTC AAT CCG TCC TAT GAC AAG ATC TTC AGT CAC CTT CAA CAT GTA	694
Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His Leu Gln His Val	
150 155 160	
AAG TAC AAA GGC CAG GAA GCA TTC GTC CCG GGA TTC AAC ATT GAA GAG	742
Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe Asn Ile Glu Glu	
165 170 175 180	
CTG CTT CCG GAG AGG ACC GCT GAA TAT TAC CGC TAC CGG GGG TCC CTG	790
Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr Arg Gly Ser Leu	
185 190 195	
ACC ACA CCC CCT TGC AAC CCC ACT GTG CTC TGG ACA GTT TTC CGA AAC	838
Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr Val Phe Arg Asn	
200 205 210	

CCC GTG CAA ATT TCC CAG GAG CAG CTG CTG GCT TTG GAG ACA GCC CTG Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu Glu Thr Ala Leu 215 220 225	886
TAC TGC ACA CAC ATG GAC GAC CCT TCC CCC AGA GAA ATG ATC AAC AAC Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu Met Ile Asn Asn 230 235 240	934
TTC CGG CAG GTC CAG AAG TTC GAT GAG AGG CTG GTA TAC ACC TCC TTC Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val Tyr Thr Ser Phe 245 250 255 260	982
TCC CAA GTG CAA GTC TGT ACT GCG GCA GGA CTG AGT CTG GGC ATC ATC Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu Ser Leu Gly Ile Ile 265 270 275	1030
CTC TCA CTG GCC CTG GCT GGC ATT CTT GGC ATC TGT ATT GTG GTG GTG Leu Ser Leu Ala Leu Ala Gly Ile Leu Gly Ile Cys Ile Val Val Val 280 285 290	1078
GTG TCC ATT TGG CTT TTC AGA AGG AAG AGT ATC AAA AAA GGT GAT AAC Val Ser Ile Trp Leu Phe Arg Arg Lys Ser Ile Lys Lys Gly Asp Asn 295 300 305	1126
AAG GGA GTC ATT TAC AAG CCA GCC ACC AAG ATG GAG ACT GAG GCC CAC Lys Gly Val Ile Tyr Lys Pro Ala Thr Lys Met Glu Thr Glu Ala His 310 315 320	1174
GCT TGAGGTCCCC GGAGCTCCCG GGCACATCCA GGAAGGACCT TGCTTTGGAC Ala 325	1227
CCTACACACT TCGGCTCTCT GGACACTTGC GACACCTCAA GGTGTTCTCT GTAGCTCAAT	1287
CTGCAACAT GCCAGGCCTC AGGGATCCTC TGCTGGGTGC CTCCTTGCCT TGGGACCATG	1347
GCCACCCCAG AGCCATCCGA TCGATGGATG GGATGCACTC TCAGACCAAG CAGCAGGAAT	1407
TCAAAGCTGC TTGCTGTAAC TGTGTGAGAT TGTGAAGTGG TCTGAATTCT GGAATCACAA	1467
ACCAAGCCAT GCTGGTGGGC CATTAAATGGT TGGAAAACAC TTTTCATCCGG GGCTTTGCCA	1527
GAGCGTGCTT TCAAGTGTC TGGAAATTCT GCTGCTTCTC CAAGCTTTCA GACAAGAATG	1587
TGCACTCTCT GCTTAGGTTT TGCTTGGGAA ACTCAACTTC TTTCTCTGG AGACGGGGCA	1647
TCTCCCTCTG ATTCCTTCT GCTATGACAA AACCTTTAAT CTGCACCTTA CAACTCGGGG	1707
ACAAATGGGG ACAGGAAGGA TCAAGTTGTA GAGAGAAAAA GAAAACAAGA GATATACATT	1767
GTGATATATT AGGGACACTT TCACAGTCCT GTCCTCTGGA TCACAGACAC TGCACAGACC	1827
TTAGGGAATG GCAGGTTCAA GTTCCACTTC TTGGTGGGGA TGAGAAGGGA GAGAGAGCTA	1887
GAGGGACAAA GAGAATGAGA AGACATGGAT GATCTGGGAG AGTCTCACTT TGGAATCAGA	1947
ATTGGAATCA CATTCTGTTT ATCAAGCCAT AATGTAAGGA CAGAATAATA CAATATTAAG	2007

TCCAAATCCA ACCTCCTGTC AGTGGAGCAG TTATGTTTTA TACTCTACAG ATTTTACAAA 2067
 TAATGAGGCT GTTCCTTGAA AATGTGTTGT TGCTGTGTCC TGGAGGAGAC ATGAGTCCG 2127
 AGATGAC 2134

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 354 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Met Pro Arg Arg Ser Leu His Ala Ala Val Leu Leu Leu Val Ile
 -29 -25 -20 -15
 Leu Lys Glu Gln Pro Ser Ser Pro Ala Pro Val Asn Gly Ser Lys Trp
 -10 -5 1
 Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys Lys Tyr Pro
 5 10 15
 Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His Ser Asp Ile
 20 25 30 35
 Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln Gly Tyr Asn
 40 45 50
 Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly His Ser Val
 55 60 65
 Lys Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu Gln Ser Arg
 70 75 80
 Tyr Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro Asn Asp Pro
 85 90 95
 His Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala Ala Glu Leu
 100 105 110 115
 His Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala Ser Thr Ala
 120 125 130
 Ser Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu Ile Glu Met
 135 140 145
 Gly Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His Leu Gln His
 150 155 160
 Val Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe Asn Ile Glu
 165 170 175

51

Glu Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr Arg Gly Ser
 180 185 190 195
 Leu Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr Val Phe Arg
 200 205 210
 Asn Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu Glu Thr Ala
 215 220 225
 Leu Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu Met Ile Asn
 230 235 240
 Asn Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val Tyr Thr Ser
 245 250 255
 Phe Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu Ser Leu Gly Ile
 260 265 270 275
 Ile Leu Ser Leu Ala Leu Ala Gly Ile Leu Gly Ile Cys Ile Val Val
 280 285 290
 Val Val Ser Ile Trp Leu Phe Arg Arg Lys Ser Ile Lys Lys Gly Asp
 295 300 305
 Asn Lys Gly Val Ile Tyr Lys Pro Ala Thr Lys Met Glu Thr Glu Ala
 310 315 320
 His Ala
 325

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 624 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: both
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CCAATCTGCC TTTGAATCTG GAGGAAATAG GCAGAAACAA AATGACTGTA GAACTTATTC	60
TCTGTAGGCC AAATTTTCATT TCAGCCACTT CTGCAGGATC CCTACTGCCA ACCTGGAATG	120
GAGACTTTTA TCTACTTCTC TCTCTCTGAA GATGTCAAAT CGTGGTTTATG ATCAAATATA	180
TTTCAAGCTA TAAAAGCAGG AGGTTATCTG TGCAGGGGGC TGGCATCATG TATTTAGGGG	240
CAAGTAATAA TGGAATGCTA CTAAGATACT CCATATTCTT CCCCGAATCA CACAGACAGT	300
TTCTGACAGG CGCAACTCCT CCATTTTCCT CCCGCAGGTG AGAACCTGT GGAGATGAGT	360
CAGTGCCATG ACTGAGAAGG AACCGACCCC TAGTTGAGAG CACCTTGAGG TTCCCCGAGA	420
ACTTTCTGAT TCACAGTCTC ATTTTGACAG CATGAAATGT CCTCTTGAAG CATAGCTTTT	480

TAAATATCTT TTTCCTTCTA CTCCTCCCTC TGACTCTAAG AATTCTCTCT TCTGGAATCG 540
CTTGAACCCA GGAGGCGGAG GTTGCACTAA GCCAAGGTCA TGCCACTGCA CTCTAGCCTG 600
GGTGACAGAG CGAGACTCCA TCTC 624

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..12

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AGA AGG AAG AGT
Arg Arg Lys Ser

12

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 4 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Arg Arg Lys Ser
1

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TGAGTCGACG

10

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AATTCGTCGA CTCA

14

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 813 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..813

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TCC AAG TGG ACT TAT TTT GGT CCT GAT GGG GAG AAT AGC TGG TCC AAG	48
Ser Lys Trp Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys	
1 5 10 15	
AAG TAC CCG TCG TGT GGG GGC CTG CTG CAG TCC CCC ATA GAC CTG CAC	96
Lys Tyr Pro Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His	
20 25 30	
AGT GAC ATC CTC CAG TAT GAC GCC AGC CTC ACG CCC CTC GAG TTC CAA	144
Ser Asp Ile Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln	
35 40 45	
GGC TAC AAT CTG TCT GCC AAC AAG CAG TTT CTC CTG ACC AAC AAT GGC	192
Gly Tyr Asn Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly	
50 55 60	

54

CAT TCA GTG AAG CTG AAC CTG CCC TCG GAC ATG CAC ATC CAG GGC CTC His Ser Val Lys Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu 65 70 75 80	240
CAG TCT CGC TAC AGT GCC ACG CAG CTG CAC CTG CAC TGG GGG AAC CCG Gln Ser Arg Tyr Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro 85 90 95	288
AAT GAC CCG CAC GGC TCT GAG CAC ACC GTC AGC GGA CAG CAC TTC GCC Asn Asp Pro His Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala 100 105 110	336
GCC GAG CTG CAC ATT GTC CAT TAT AAC TCA GAC CTT TAT CCT GAC GCC Ala Glu Leu His Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala 115 120 125	384
AGC ACT GCC AGC AAC AAG TCA GAA GGC CTC GCT GTC CTG GCT GTT CTC Ser Thr Ala Ser Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu 130 135 140	432
ATT GAG ATG GGC TCC TTC AAT CCG TCC TAT GAC AAG ATC TTC AGT CAC Ile Glu Met Gly Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His 145 150 155 160	480
CTT CAA CAT GTA AAG TAC AAA GGC CAG GAA GCA TTC GTC CCG GGA TTC Leu Gln His Val Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe 165 170 175	528
AAC ATT GAA GAG CTG CTT CCG GAG AGG ACC GCT GAA TAT TAC CGC TAC Asn Ile Glu Glu Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr 180 185 190	576
CGG GGG TCC CTG ACC ACA CCC CCT TGC AAC CCC ACT GTG CTC TGG ACA Arg Gly Ser Leu Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr 195 200 205	624
GTT TTC CGA AAC CCC GTG CAA ATT TCC CAG GAG CAG CTG CTG GCT TTG Val Phe Arg Asn Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu 210 215 220	672
GAG ACA GCC CTG TAC TGC ACA CAC ATG GAC GAC CCT TCC CCC AGA GAA Glu Thr Ala Leu Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu 225 230 235 240	720
ATG ATC AAC AAC TTC CGG CAG GTC CAG AAG TTC GAT GAG AGG CTG GTA Met Ile Asn Asn Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val 245 250 255	768
TAC ACC TCC TTC TCC CAA GTG CAA GTC TGT ACT GCG GCA GGA CTG Tyr Thr Ser Phe Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu 260 265 270	813

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 271 amino acids

(B) TYPE: amino acid

55

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

```

Ser Lys Trp Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys
 1           5           10           15
Lys Tyr Pro Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His
          20           25           30
Ser Asp Ile Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln
          35           40           45
Gly Tyr Asn Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly
          50           55           60
His Ser Val Lys Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu
          65           70           75           80
Gln Ser Arg Tyr Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro
          85           90           95
Asn Asp Pro His Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala
          100          105          110
Ala Glu Leu His Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala
          115          120          125
Ser Thr Ala Ser Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu
          130          135          140
Ile Glu Met Gly Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His
          145          150          155          160
Leu Gln His Val Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe
          165          170          175
Asn Ile Glu Glu Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr
          180          185          190
Arg Gly Ser Leu Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr
          195          200          205
Val Phe Arg Asn Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu
          210          215          220
Glu Thr Ala Leu Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu
          225          230          235          240
Met Ile Asn Asn Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val
          245          250          255
Tyr Thr Ser Phe Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu
          260          265          270

```

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 822 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: both
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..822

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TCC AAG TGG ACT TAT TTT GGT CCT GAT GGG GAG AAT AGC TGG TCC AAG	48
Ser Lys Trp Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys	
1 5 10 15	
AAG TAC CCG TCG TGT GGG GGC CTG CTG CAG TCC CCC ATA GAC CTG CAC	96
Lys Tyr Pro Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His	
20 25 30	
AGT GAC ATC CTC CAG TAT GAC GCC AGC CTC ACG CCC CTC GAG TTC CAA	144
Ser Asp Ile Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln	
35 40 45	
GGC TAC AAT CTG TCT GCC AAC AAG CAG TTT CTC CTG ACC AAC AAT GGC	192
Gly Tyr Asn Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly	
50 55 60	
CAT TCA GTG AAG CTG AAC CTG CCC TCG GAC ATG CAC ATC CAG GGC CTC	240
His Ser Val Lys Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu	
65 70 75 80	
CAG TCT CGC TAC AGT GCC ACG CAG CTG CAC CTG CAC TGG GGG AAC CCG	288
Gln Ser Arg Tyr Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro	
85 90 95	
AAT GAC CCG CAC GGC TCT GAG CAC ACC GTC AGC GGA CAG CAC TTC GCC	336
Asn Asp Pro His Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala	
100 105 110	
GCC GAG CTG CAC ATT GTC CAT TAT AAC TCA GAC CTT TAT CCT GAC GCC	384
Ala Glu Leu His Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala	
115 120 125	
AGC ACT GCC AGC AAC AAG TCA GAA GGC CTC GCT GTC CTG GCT GTT CTC	432
Ser Thr Ala Ser Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu	
130 135 140	
ATT GAG ATG GGC TCC TTC AAT CCG TCC TAT GAC AAG ATC TTC AGT CAC	480
Ile Glu Met Gly Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His	
145 150 155 160	

57

CTT CAA CAT GTA AAG TAC AAA GGC CAG GAA GCA TTC GTC CCG GGA TTC	528
Leu Gln His Val Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe	
165 170 175	
AAC ATT GAA GAG CTG CTT CCG GAG AGG ACC GCT GAA TAT TAC CGC TAC	576
Asn Ile Glu Glu Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr	
180 185 190	
CGG GGG TCC CTG ACC ACA CCC CCT TGC AAC CCC ACT GTG CTC TGG ACA	624
Arg Gly Ser Leu Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr	
195 200 205	
GTT TTC CGA AAC CCC GTG CAA ATT TCC CAG GAG CAG CTG CTG GCT TTG	672
Val Phe Arg Asn Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu	
210 215 220	
GAG ACA GCC CTG TAC TGC ACA CAC ATG GAC GAC CCT TCC CCC AGA GAA	720
Glu Thr Ala Leu Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu	
225 230 235 240	
ATG ATC AAC AAC TTC CGG CAG GTC CAG AAG TTC GAT GAG AGG CTG GTA	768
Met Ile Asn Asn Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val	
245 250 255	
TAC ACC TCC TTC TCC CAA GTG CAA GTC TGT ACT GCG GCA GGA CTG AGT	816
Tyr Thr Ser Phe Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu Ser	
260 265 270	
CTG GGC	822
Leu Gly	

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 274 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Ser Lys Trp Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys	
1 5 10 15	
Lys Tyr Pro Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His	
20 25 30	
Ser Asp Ile Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln	
35 40 45	
Gly Tyr Asn Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly	
50 55 60	
His Ser Val Lys Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu	
65 70 75 80	

58

[illegible]

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 25 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(11) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CTTTTTTGAT ACCCTTCCTT CTGAA

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 986 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: both
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..975

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

TCC AAG TGG ACT TAT TTT GGT CCT GAT GGG GAG AAT AGC TGG TCC AAG	48
Ser Lys Trp Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys	
1 5 10 15	
AAG TAC CCG TCG TGT GGG GGC CTG CTG CAG TCC CCC ATA GAC CTG CAC	96
Lys Tyr Pro Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His	
20 25 30	
AGT GAC ATC CTC CAG TAT GAC GCC AGC CTC ACG CCC CTC GAG TTC CAA	144
Ser Asp Ile Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln	
35 40 45	
GGC TAC AAT CTG TCT GCC AAC AAG CAG TTT CTC CTG ACC AAC AAT GGC	192
Gly Tyr Asn Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly	
50 55 60	
CAT TCA GTG AAG CTG AAC CTG CCC TCG GAC ATG CAC ATC CAG GGC CTC	240
His Ser Val Lys Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu	
65 70 75 80	
CAG TCT CGC TAC AGT GCC ACG CAG CTG CAC CTG CAC TGG GGG AAC CCG	288
Gln Ser Arg Tyr Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro	
85 90 95	
AAT GAC CCG CAC GGC TCT GAG CAC ACC GTC AGC GGA CAG CAC TTC GCC	336
Asn Asp Pro His Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala	
100 105 110	
GCC GAG CTG CAC ATT GTC CAT TAT AAC TCA GAC CTT TAT CCT GAC GCC	384
Ala Glu Leu His Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala	
115 120 125	
AGC ACT GCC AGC AAC AAG TCA GAA GGC CTC GCT GTC CTG GCT GTT CTC	432
Ser Thr Ala Ser Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu	
130 135 140	
ATT GAG ATG GGC TCC TTC AAT CCG TCC TAT GAC AAG ATC TTC AGT CAC	480
Ile Glu Met Gly Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His	
145 150 155 160	

60

CTT CAA CAT GTA AAG TAC AAA GGC CAG GAA GCA TTC GTC CCG GGA TTC Leu Gln His Val Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe 165 170 175	528
AAC ATT GAA GAG CTG CTT CCG GAG AGG ACC GCT GAA TAT TAC CGC TAC Asn Ile Glu Glu Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr 180 185 190	576
CGG GGG TCC CTG ACC ACA CCC CCT TGC AAC CCC ACT GTG CTC TGG ACA Arg Gly Ser Leu Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr 195 200 205	624
GTT TTC CGA AAC CCC GTG CAA ATT TCC CAG GAG CAG CTG CTG GCT TTG Val Phe Arg Asn Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu 210 215 220	672
GAG ACA GCC CTG TAC TGC ACA CAC ATG GAC GAC CCT TCC CCC AGA GAA Glu Thr Ala Leu Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu 225 230 235 240	720
ATG ATC AAC AAC TTC CGG CAG GTC CAG AAG TTC GAT GAG AGG CTG GTA Met Ile Asn Asn Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val 245 250 255	768
TAC ACC TCC TTC TCC CAA GTG CAA GTC TGT ACT GCG GCA GGA CTG AGT Tyr Thr Ser Phe Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu Ser 260 265 270	816
CTG GGC ATC ATC CTC TCA CTG GCC CTG GCT GGC ATT CTT GGC ATC TGT Leu Gly Ile Ile Leu Ser Leu Ala Leu Ala Gly Ile Leu Gly Ile Cys 275 280 280	864
ATT GTG GTG GTG GTG TCC ATT TGG CTT TTC AGA AGG AAG GGT ATC AAA Ile Val Val Val Val Ser Ile Trp Leu Phe Arg Arg Lys Gly Ile Lys 290 295 300	912
AAA GGT GAT AAC AAG GGA GTC ATT TAC AAG CCA GCC ACC AAG ATG GAG Lys Gly Asp Asn Lys Gly Val Ile Tyr Lys Pro Ala Thr Lys Met Glu 305 310 315 320	960
ACT GAG GCC CAC GCT TGAGGTCCCC G Thr Glu Ala His Ala 325	986

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 325 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Ser Lys Trp Thr Tyr Phe Gly Pro Asp Gly Glu Asn Ser Trp Ser Lys
1 5 10 15

61

Lys Tyr Pro Ser Cys Gly Gly Leu Leu Gln Ser Pro Ile Asp Leu His
 20 25 30
 Ser Asp Ile Leu Gln Tyr Asp Ala Ser Leu Thr Pro Leu Glu Phe Gln
 35 40 45
 Gly Tyr Asn Leu Ser Ala Asn Lys Gln Phe Leu Leu Thr Asn Asn Gly
 50 55 60
 His Ser Val Lys Leu Asn Leu Pro Ser Asp Met His Ile Gln Gly Leu
 65 70 75 80
 Gln Ser Arg Tyr Ser Ala Thr Gln Leu His Leu His Trp Gly Asn Pro
 85 90 95
 Asn Asp Pro His Gly Ser Glu His Thr Val Ser Gly Gln His Phe Ala
 100 105 110
 Ala Glu Leu His Ile Val His Tyr Asn Ser Asp Leu Tyr Pro Asp Ala
 115 120 125
 Ser Thr Ala Ser Asn Lys Ser Glu Gly Leu Ala Val Leu Ala Val Leu
 130 135 140
 Ile Glu Met Gly Ser Phe Asn Pro Ser Tyr Asp Lys Ile Phe Ser His
 145 150 155 160
 Leu Gln His Val Lys Tyr Lys Gly Gln Glu Ala Phe Val Pro Gly Phe
 165 170 175
 Asn Ile Glu Glu Leu Leu Pro Glu Arg Thr Ala Glu Tyr Tyr Arg Tyr
 180 185 190
 Arg Gly Ser Leu Thr Thr Pro Pro Cys Asn Pro Thr Val Leu Trp Thr
 195 200 205
 Val Phe Arg Asn Pro Val Gln Ile Ser Gln Glu Gln Leu Leu Ala Leu
 210 215 220
 Glu Thr Ala Leu Tyr Cys Thr His Met Asp Asp Pro Ser Pro Arg Glu
 225 230 235 240
 Met Ile Asn Asn Phe Arg Gln Val Gln Lys Phe Asp Glu Arg Leu Val
 245 250 255
 Tyr Thr Ser Phe Ser Gln Val Gln Val Cys Thr Ala Ala Gly Leu Ser
 260 265 270
 Leu Gly Ile Ile Leu Ser Leu Ala Leu Ala Gly Ile Leu Gly Ile Cys
 275 280 285
 Ile Val Val Val Val Ser Ile Trp Leu Phe Arg Arg Lys Gly Ile Lys
 290 295 300
 Lys Gly Asp Asn Lys Gly Val Ile Tyr Lys Pro Ala Thr Lys Met Glu
 305 310 315 320

Thr Glu Ala His Ala
325

(2) INFORMATION FOR SEQ ID NO:19:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ACATTGAAGA GCTGCTTCGG G

21

(2) INFORMATION FOR SEQ ID NO:20:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

AATTTCACG GGGTTTCGG

19

(2) INFORMATION FOR SEQ ID NO:21:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1363 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

CTGACACCAC TCAGACCGTG TGTGATCTGG CTCAACCACT TCTGCGATCC CACCCAGGAA	60
CAGAAGACTG CAAGAAAACG TTAATTCAAC CCCCTGTGA TCCCATCTGC AACCTGACCA	120
ATCAGCACTC CCCAAGTCCC AAGCCCCTAT CTGCCAAATT ATCTTTAAAA ACTCCCCAGA	180
GGCAGGGTGC AGTGGTTCAA CGCCTGTAAT CCCAGCACTT TAGGTGGATC ACGAGATCAA	240
GAGATCAAGA CCAGCCTGGC CAACATGGTG AAACCCCGTC TTCTTACTAA AAATACAAAA	300
ATTAGCTGGG TGTGGCGGCG CGTGCCTGTA ATCCCAGCTA CCCAGGAGGC TGAGGCAGGA	360

63

GAATCGCTTG AACCCGTGAG GCAGAGGTTG CAGTGAGCCA AGACCATGCC ACTGCATTTT	420
AGCCTGGGCG ACAGAGGGGA ACTCCGTCTG AACAAACAAA CAAACAAACA ACTCCCGGAA	480
TGCTTGGGGA GACTGATTTG AGTACTGGAA TCCCAGTACT TTAGGAGGCC AAGGTAGGTG	540
GATCATTTGA GGTGAGGAGT TCCAGACCAG CCTGGCCAAC ATGGTGAAAC CCCGTCTCTA	600
CTAAAATTAG AAAAATTAGC CGGGTGTGGT GGTGGGCGCC TGTAAATCCA GCACTTTGGG	660
AAGCCAAGGC AGGTGAATTA TCTGAGGTCG GGAGTTTAAG GCCAGCCTTA AACTGGCGAA	720
ACCCCGCCTC TACTAAAAAT ACAAAAATTA TCTGGGCATG GTGGCATGTG CCTGTAATCC	780
CAGCTACTCG GGAGGCTGAG GCAGGAGAAT CGCTTGAACC CGGGAGGCGG AGGTTGCACT	840
GAGCCGAGAT CACGCTATTG CACTCCGGCC TGGGCAACAG AGCGAGACTC CGTCTCAAAC	900
AAACAAACAA AGGAACGAAA ACTCCGGTCT CCGGCACGGC AAGCTCTGCG TGAATTACTT	960
TCTCCATTGC AACTCCCCTG TCTTGATAAA TGGGCTCTGT CTAAGCAGCG GGCAAGGTGA	1020
ACTCGTTGGG CTGTTACAGG ACCAGTGACA GACCAAGGCA TGCCACTGAA GGAATCCCTA	1080
GACGCACCCT TCTGGATGTG AGGCAGGCGG ATCTCACCCC ACGCCTGCCA GCAGCTCCTC	1140
GGAGAACTGT GTTCCTGGGT CAGCCCTGGC CCAGAGGAGC GCCGGGGACC CGCAGAGTGC	1200
TGCTGAAGTC AAGGCTACAA CTCACCTAGG ATCTGGGGCG CCAGCCTCCG GTGGGCAGGG	1260
CGTTCTCCTC CCCACCCCC TCCCCGCACG ATGACATCAA GTGTTTGGCG TTGAGTTGCT	1320
CCATAAAAGC TGCCCGGGGA AGCCAGGAGA GCGAAGGGCG GAC	1363

(2) INFORMATION FOR SEQ ID NO:22:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GTCCACTTGG ATCCGTTTAC TGG

WE CLAIM:

1. A substantially purified nucleic acid encoding the amino acid sequence of HCAVIII depicted in SEQ ID NO:2.

2. The nucleic acid of Claim 1 wherein said nucleic acid is mRNA.

3. A cDNA encoding the amino acid sequence of HCAVIII or a portion thereof.

4. The cDNA of Claim 3 wherein the amino acid sequence is encoded by the coding region of the nucleotide sequence depicted in SEQ ID NO:1.

5. The cDNA of Claim 3 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:2.

6. The cDNA of Claim 3 wherein the amino acid sequence is encoded by the coding region of the nucleotide sequence depicted in SEQ ID NO:3.

7. The cDNA of Claim 3 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:4.

8. The cDNA of Claim 3 wherein the amino acid sequence is encoded by the nucleotide sequence depicted in SEQ ID NO:12.

9. The cDNA of Claim 3 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:13.

10. The cDNA of Claim 3 wherein the amino acid sequence is encoded by the nucleotide sequence depicted in SEQ ID NO:14.

11. The cDNA of Claim 3 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:15.

12. The cDNA of Claim 3 comprising the nucleotide sequence depicted in SEQ ID NO:5.

13. The cDNA of Claim 3 comprising the nucleotide sequences depicted in SEQ ID NO:5 and SEQ ID NO:7.

14. A cDNA encoding the amino acid sequence of HCAVIII wherein the phosphorylation region has been mutated.

15. The cDNA of Claim 14 wherein the amino acid sequence is encoded by the nucleic acid sequence depicted in SEQ ID NO:17.

16. The cDNA of Claim 14 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:18.

17. A protein comprising the amino acid sequence of HCAVIII or a portion thereof.

18. The protein of Claim 17 wherein the amino acid sequence is encoded by the coding region of the nucleic acid sequence depicted in SEQ ID NO:1.

19. The protein of Claim 17 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:2.

20. The protein of Claim 17 wherein the amino acid sequence is encoded by the coding region of the nucleic acid sequence depicted in SEQ ID NO:3.

21. The protein of Claim 17 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:4.

22. The protein of Claim 17 wherein the amino acid sequence is encoded by the coding region of the nucleic acid sequence depicted in SEQ ID NO:12.

23. The protein of Claim 17 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:13.

24. The protein of Claim 17 wherein the amino acid sequence is encoded by the coding region of the nucleic acid sequence depicted in SEQ ID NO:14.

25. The protein of Claim 17 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:15.

26. A protein comprising the amino acid sequence of HCAVIII wherein the phosphorylation region has been mutated.

27. The protein of claim 26 wherein the amino acid sequence is encoded by the nucleic acid sequence depicted in SEQ ID NO:17.

28. The protein of Claim 26 wherein the amino acid sequence comprises the sequence depicted in SEQ ID NO:18.

29.. A recombinant DNA clone comprising a cDNA of a HCAVIII transcript isolatable from human A549 cells of about 1.1 kilobases.

30. An expression vector comprising the nucleic sequence for HCAVIII or a portion thereof.

31. The expression vector of Claim 30 wherein the nucleic acid sequence comprises the coding region of the nucleotide sequence depicted in SEQ ID NO:1.

32. The expression vector of Claim 30 wherein the nucleic acid sequence comprises the coding region of the nucleotide sequence depicted in SEQ ID NO:3

33. The expression vector of Claim 30 wherein the nucleic acid sequence comprises the coding region of the nucleotide sequence depicted in SEQ ID NO:12.

34. The expression vector of Claim 30 wherein the nucleic acid sequence comprises the coding region of the nucleotide sequence depicted in SEQ ID NO:14.

35. The expression vector of Claim 30 wherein the nucleic acid sequence comprises the nucleotide sequence depicted in SEQ ID NO:17.

- 5 36. A method of detecting cancerous and precancerous
lung tissue comprising:
- (a) preparing a section of biopsy tissue;
 - (b) probing said tissue with a labeled probe
complementary to the cDNA of SEQ ID NO:1;
 - 10 (c) removing said probe which has not hybridized to
the tissue; and
 - (d) detecting the presence of the hybridized probe.

37. A method for detecting lung cancer antigen specific for non-small cell carcinoma in a human cell specimen comprising:

a) labeling a DNA probe comprising the genomic clone of HCAVIII;

5 b) reacting the labeled DNA probe with a human test cell specimen and a normal human cell specimen under conditions suitable for hybridization of the labeled probe to any HCAVIII mRNA which may be present in the test and normal cell specimen;

10 c) removing unreacted components from the test and said normal cell specimens;

 d) detecting the hybridized probe bound to the test and normal cell specimens;

15 e) quantifying and comparing the amount of hybridized probe bound to the test and normal cell specimens.

38. The method of claim 37 further comprising:

a) labeling a DNA probe comprising the genomic clone of HCAVIII with a substrate which can bind to a detecting substance to form a labeled DNA probe;

5 b) reacting the labeled DNA probe with a human test cell specimen and a normal human cell specimen under conditions suitable for hybridization of the labeled probe to any HCAVIII mRNA which may be present in the test and normal cell specimens;

10 c) removing unreacted components from the test and normal cell specimens;

 d) reacting the test and normal cell specimens with a detecting substance which is capable of fluorescing;

 e) comparing the fluorescence of the test and normal cell specimens.

39. A method for screening human specimens for HCAVIII protein, comprising:

- 5 a) mixing a human test specimen with a first amount of an antibody specific for the HCAVIII protein in a first reaction well;
- b) mixing a control lung cancer antigen comprising at least a portion of the HCAVIII protein with a second amount of said antibody specific for the HCAVIII protein in a second reaction well; and
- 10 c) detecting whether said test specimen binds to said antibody as compared to said control lung cancer antigen.

40. A method for testing a human cell sample for lung cancer comprising assaying a cell homogenate for carbonic anhydrase activity.

41. An antibody made by immunizing animals with a lung cancer antigen associated with non-small cell lung cancer cells.

42. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:2.

43. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:4.

44. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:13.

45. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:15.

46. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:18.

47. A therapeutic composition for the treatment of non-small cell lung cancer comprising an antibody to HCAVIII protein bound to a substance which affects the ability of said cancer to replicate.

48. The method of claim 47 wherein said substance is a cancer drug.

49. The method of claim 48 wherein said substance is a radioisotope.

50. The method of claim 49 wherein said substance affects gene expression of a gene encoding HCAVIII.

51. A substantially purified nucleic acid comprising the nucleotide sequence depicted in SEQ ID NO:7.

52. A cDNA comprising the nucleotide sequence depicted in SEQ ID NO:7.

53. A substantially purified nucleic acid comprising the nucleotide sequence depicted in SEQ ID NO:21.

AMENDED CLAIMS

[received by the International Bureau on 20 November 1995 (20.11.95);
original claim 41 amended; remaining claims unchanged (1 page)]

41. An antibody made by immunizing animals with HCAVIII, a lung cancer antigen associated with non-small cell lung cancer cells.

42. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:2.

43. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:4.

44. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:13.

45. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:15.

46. The antibody of Claim 41 wherein said lung cancer antigen has the amino acid sequence depicted in SEQ ID NO:18.

[illegible]

INTERNATIONAL SEARCH REPORT

In: ational application No.
PCT/US95/09145

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : Please See Extra Sheet.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/22.1, 23.1; 530/350, 387.1; 435/4, 6, 7.1, 7.2; 514/44, 424/85.8

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	American Journal of Pathology, Volume 142, Number 1, issued January 1993, Kim et al, "Interphase cytogenetics in paraffin sections of lung tumors by non-isotopic in situ hybridization", pages 307-317.	36-38
A	Cancer (supplement), Volume 69, Number 6, issued 15 March 1992, Gray et al, "Molecular Cytogenetics in Human Cancer Diagnosis", pages 1536-1542.	36-38
A	Cancer Research (Supplement), Volume 52, issue 01 May 1992, Birrer et al, "Application of molecular genetics to the early diagnosis and screening of lung cancer", pages 2658s-2664s.	36-40

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	* T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
* A		document defining the general state of the art which is not considered to be of particular relevance
* E		earlier document published on or after the international filing date
* L		documents which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
* O		document referring to an oral disclosure, use, exhibition or other means
* P		document published prior to the international filing date but later than the priority date claimed
	* X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
	* Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
	* A	document member of the same patent family

Date of the actual completion of the international search 12 OCTOBER 1995	Date of mailing of the international search report 25 OCT 1995
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer <i>Dorothy Frieser for</i> ETHAN WHISENANT Telephone No. (703) 308-1096

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/09145

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US,A, 5,134,075 (HELLSTROM ET AL) 28 July 1992, especially column 4 lines 34-64.	39, 41, 47-50
Y	US,A, 4,816,402 (ROSEN ET AL) 28 March 1989, see entire document.	39, 41
A	Gastroenterology, Volume 105, Number 3, issued 1993, Mori et al, "The significance of carbonic anhydrase expression in human colorectal cancer", pages 820-826, see abstract.	40
A	DNA and Cell Biology, Volume 11, Number 7, issued September 1992, Skonier et al, "cDNA cloning and sequence analysis of Big-h3, a novel gene induced in a human adenocarcinoma cell line after treatment with transforming growth factor-beta", pages 511-522, see entire document.	1-35, 42-46, 51-53

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/09145

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US95/09145

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (6):

C07H 19/00, 21/00, 21/02, 21/04; C07K 1/00, 14/00, 17/00, 16/00; C12Q 1/00, 1/68; G01N 33/53, 33/567; A01N 43/04; A61K 31/70

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

536/22.1, 23.1; 530/350, 387.1; 435/4, 6, 7.1, 7.2; 514/44, 424/85.8

B. FIELDS SEARCHED

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, BIOSIS, CAPLUS, CANCERLIT

search terms: AS49, HCAVIII, Human Cancer Antigen VIII, Cell surface antigen, Cell surface marker, Non-small cell lung cancer, Carbonic Anhydrase

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I, claim(s) 1-39 and 41-53, drawn to nucleic acids encoding the amino acid sequence of HCVIII, the protein expressed thereof, antibodies to the proteins and methods using one of the above.

Group II, claim(s) 40, drawn to a method for testing a human cell sample for lung cancer by assaying a cell homogenate for carbonic anhydrase activity.

Pursuant to 37 CFR § 1.475(d) the additional method(s) beyond the one first method of use are considered to lack unity and are properly separated.